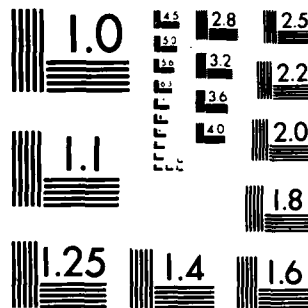


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REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER AFOSR-TR- 84-0118	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Sublethal Effects of JP-4 on Aquatic Organisms and Communities		5. TYPE OF REPORT & PERIOD COVERED Annual Report 1 Nov 82 - 31 Oct 83
6. AUTHOR(s) Cairns, John Jr. Buikema, Arthur L. Jr. Doane, Thomas R. Neiderlehner, R. R.		6. PERFORMING ORG. REPORT NUMBER
7. PERFORMING ORGANIZATION NAME AND ADDRESS University Center for Environmental Studies Virginia Polytechnic Institute and State Univ. Blacksburg, Virginia 24061		8. CONTRACT OR GRANT NUMBER(s) AFOSR-82-0059
9. CONTROLLING OFFICE NAME AND ADDRESS AFOSR/NL Bolling AFB, Washington DC 20332		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 2312/45 61102F
11. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		12. REPORT DATE January 1984
		13. NUMBER OF PAGES 64
		14. SECURITY CLASS. (of this report) Unclassified
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

DISTRIBUTION STATEMENT (of this Report)

Approved for public release;
 distribution unlimited.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)		
18. SUPPLEMENTARY NOTES		
19. KEY WORDS (Continue on reverse side if necessary and identify by block number)		
JP-4	Ventilatory rate	<u>Tanytarus dissimilis</u>
Jet Fuel	preference/avoidance	microbial community
Sublethal Effects	<u>Aeolosoma headley</u>	
<u>Lepomis macrochirus</u>	<u>Daphnia pulex</u>	
	<u>Paratanytarus narthogentica</u>	
20. ABSTRACT (Continue on reverse side if necessary and identify by block number)		
<p>In the second year of the AFOSR grant to examine the sublethal effects of water soluble fraction (WSF) of JP-4 jet fuel we have completed most of the work on the petroleum derived JP-4. Fractionators have been built and used to generate constant concentrations of the WSF JP-4 that were used to determine the lethal and sublethal effects on bluegill sunfish (<u>Lepomis macrochirus</u>) and selected aquatic invertebrates. The dynamic 96 hour LC50 for the WSF JP-4 for the bluegill was determined to be 26.2%. (This is percent of the maximum</p>		

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soluble amount of JP-4.) The concentration of the WSF JP-4 which causes a detectable shift in the ventilatory functions (rate and amplitude) was determined to be 5.1% WSF. This was approximately 20% of the nominal 96 hour LC50 and equivalent to the 96 hour LC01; this disparity was due to the steepness of the lethality curve for the jet fuel. The fish did not display a strong preference/avoidance reaction when exposed to the WSF JP-4. However, what response did occur was at concentrations that were slightly lower than the concentration at which the fish altered their ventilatory pattern. At higher concentrations the fish seemed to lose their ability to detect the WSF JP-4.

There were few significant changes in the whole and serum blood parameters that were measured after the fish were exposed to sublethal levels of the WSF JP-4. At near lethal concentrations the only pronounced effects on these parameters may primarily be attributed to osmoregulatory failure.

There were no significant effects on histology when the fish were examined with light and electron microscopy after exposure to sublethal concentrations. The only obvious difference was the apparent sequestering of metabolized JP-4 component materials in the liver. These tissues and those from fish exposed to near lethal levels will be examined more thoroughly in the next year of research.

In the second year of research producing cultures of aquatic invertebrates were established, flow-through test systems were designed and constructed, and toxicity tests with the water soluble fraction (WSF) of petroleum JP-4 were begun with 3 invertebrates, the oligochaete, Aeolosoma headleyi, a benthic collector gatherer; the cladoceran Daphnia pulex, a planktonic filter-feeding crustacean; and, the dipteran Paratanytarsus parthogentica (Freeman) (= Tanytarsus dissimilis Joh.), a substrate associated collector gatherer. An LC-50 could be determined only for Paratanytarsus, the most sensitive organism tested, and it was 2.2% WSF. There was little or no mortality in acute tests with Aeolosoma and Daphnia. Effects of chronic exposure on Aeolosoma were observed at 8.9% WSF and effects on Paratanytarsus were apparent at 3.9%. Studies exposing naturally derived freshwater microbial communities to jet fuel WSF also have been initiated. There were no significant changes in the number of species on artificial substrates after an acute exposure to 35.9% WSF. Chronic exposures to concentrations as low as 0.3% WSF may increase the colonization rate of barren substrates. This is consistent with any mild organic enrichment.



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PART I

I. INTRODUCTION

A. This is the second annual report for AFOSR Grant 82-0059. It is divided up into four major sections. Part I is an overall introduction to the entire report. An abstract is also contained in this section. Part II deals with the effects of the water soluble fraction (WSF) of JP-4 jet fuel on the bluegill sunfish (Lepomis macrochirus Raf.). The effects of the WSF JP-4 on invertebrates are discussed in Part III. The last major section, Part IV, serves as the administrative section of the report. The goals for the last year of the research are also discussed in Part IV.

B. Abstract

In the second year of the AFOSR grant to examine the sublethal effects of the water soluble fraction (WSF) of JP-4 jet fuel we have completed most of the work on the petroleum derived JP-4. Fractionators have been built and used to generate constant concentrations of the WSF JP-4 that were used to determine the lethal and sublethal effects on bluegill sunfish (Lepomis macrochirus) and selected aquatic invertebrates. The dynamic 96 hr LC50 for the WSF JP-4 for the bluegill was determined to be 26.2%. (This is percent of the maximum soluble amount of JP-4.) The concentration of the WSF JP-4 which causes a detectable shift in the ventilatory functions (rate and amplitude) was

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MATTHEW J. KERPER
Chief, Technical Information Division

determined to be 5.1 % WSF. This was approximately 20% of the nominal 96 hr LC50 and equivalent to the 96 hr LC01; this disparity was due to the steepness of the lethality curve for the jet fuel. The fish did not display a strong preference/avoidance reaction when exposed to the WSF JP-4. However, what response did occur was at concentrations that were slightly lower than the concentration at which the fish altered their ventilatory pattern. At higher concentrations the fish seemed to lose their ability to detect the WSF JP-4.

There were few significant changes in the whole and serum blood parameters that were measured after the fish were exposed to sublethal levels of the WSF JP-4. At near lethal concentrations the only pronounced effects on these parameters may primarily be attributed to osmoregulatory failure.

There were no significant effects on histology when the fish were examined with light and electron microscopy after exposure to sublethal concentrations. The only obvious difference was the apparent sequestering of metabolized JP-4 component materials in the liver. These tissues and those from fish exposed to near lethal levels will be examined more thoroughly in the next year of research.

In the second year of research reproducing cultures of aquatic invertebrates were established, flow-through test systems were designed and constructed, and toxicity tests with the water soluble fraction (WSF) of petroleum JP-4 were

begun with 3 invertebrates, the oligochaete, Aeolosoma headleyi, a benthic collector gatherer; the cladoceran Daphnia pulex, a planktonic filter-feeding crustacean; and, the dipteran Paratanytarsus parthogenetica (Freeman) (= Tanytarsus dissimilis Joh.), a substrate associated collector gatherer. An LC50 could be determined only for Paratanytarsus, the most sensitive organism tested, and it was 2.2 % WSF. There was little or no mortality in acute tests with Aeolosoma and Daphnia. Effects of chronic exposure on Aeolosoma were observed at 8.9 % WSF and effects on Paratanytarsus were apparent at 3.9 %. Studies exposing naturally derived freshwater microbial communities to jet fuel WSF also have been initiated. There were no significant changes in the number of species on artificial substrates after an acute exposure to 35.9 % WSF. Chronic exposures to concentrations as low as 0.3 % WSF may increase the colonization rate of barren substrates. This is consistent with any mild organic enrichment.

PART II

I. INTRODUCTION

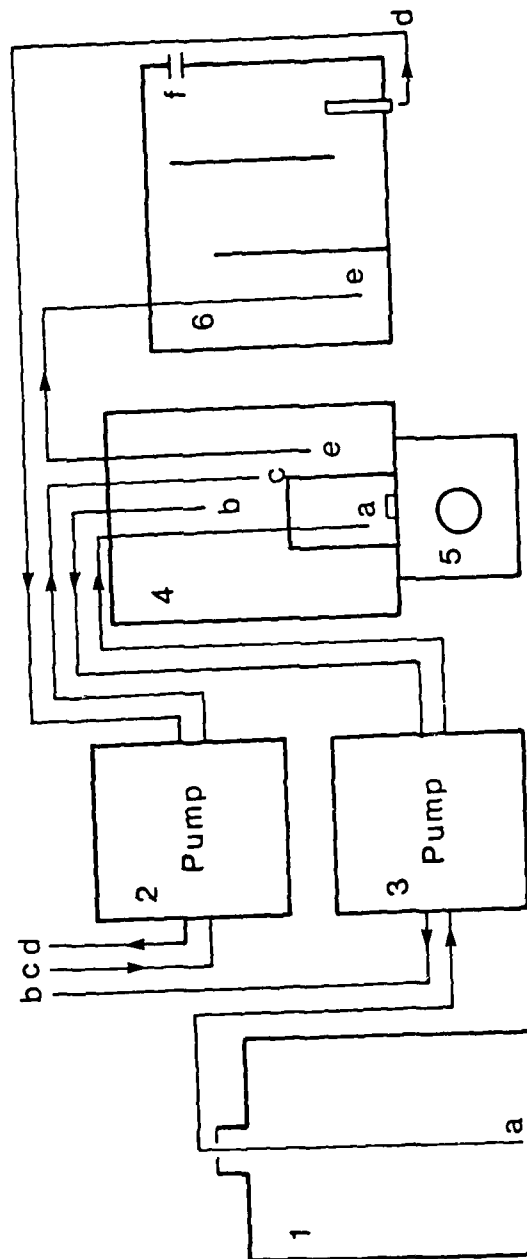
This is Part II of the second annual report for AFOSR Grant 82-0059 and deals with the sublethal effects of the water soluble fraction (WSF) of JP-4 jet fuel on the bluegill sunfish (Lepomis macrochirus).

An abstract of the results of this research can be found in Part I of report.

II. MATERIALS AND METHODS

A. Manufacture and Analysis of Water Soluble Fraction (WSF)

1. The first problem addressed was to develop a system that would generate a constant concentration of a WSF of the jet fuel (JP-4) for subsequent research. We designed and built a flow-through system consisting of a mixing chamber, settling chamber and accompanying pumps for jet fuel and water (Figure 1). The mixing chamber is a glass battery jar with a smaller beaker permanently attached in its center. The mixing chamber is set on a magnetic stirrer with the stirring bar placed in the inner beaker. Jet fuel is pumped into the beaker at approximately 5% volume to volume ratio with charcoal filtered tap water. "Spent" jet fuel is removed from the floating fuel layer at the top of the mixing chamber at the same rate that fresh jet fuel is delivered to the system. (The exact pumping rates depend on the quantity of WSF needed for each experiment).



WATER SOLUBLE FRACTIONATOR

Figure 1: Schematic of JP-4 Water Soluble Fractionator

Air is injected into the beaker to aid in mixing and oxygenation of the mixture. The mixing time is approximately one hour (depending on the flow rate of fuel and water). The WSF flows from the mixing chamber to the settling chamber where any entrained jet fuel is separated. The settling chamber is a rectangular glass box with baffles to avoid direct flow through of entrained fuel. The detention time in this chamber is also approximately one hour. The WSF flows by gravity, or is pumped, to the experimental equipment. The entire fractionator is contained in a tub that will contain any leaks resulting from pump or chamber failure. The excess WSF from the settling chamber and the outflow from the containing tub are diverted to the laboratory drain. The fractionator used for the fish test is capable of delivering 150 ml min^{-1} of WSF. The smaller fractionator used for the invertebrate tests can deliver 40 ml min^{-1} .

2. The WSF of the jet fuel is analyzed using a technique modified from one developed by the EPA. The basic concept is that the jet fuel components are extracted from the WSF with hexadecane which is then used as a carrier for injection into a gas chromatograph. A sample of the WSF is collected in a 100 ml volumetric flask and 1 ml of hexadecane is added. The flask is vigorously shaken for three minutes and then allowed to settle for another three minutes. The hexadecane layer is then removed and analyzed by gas chromatography.

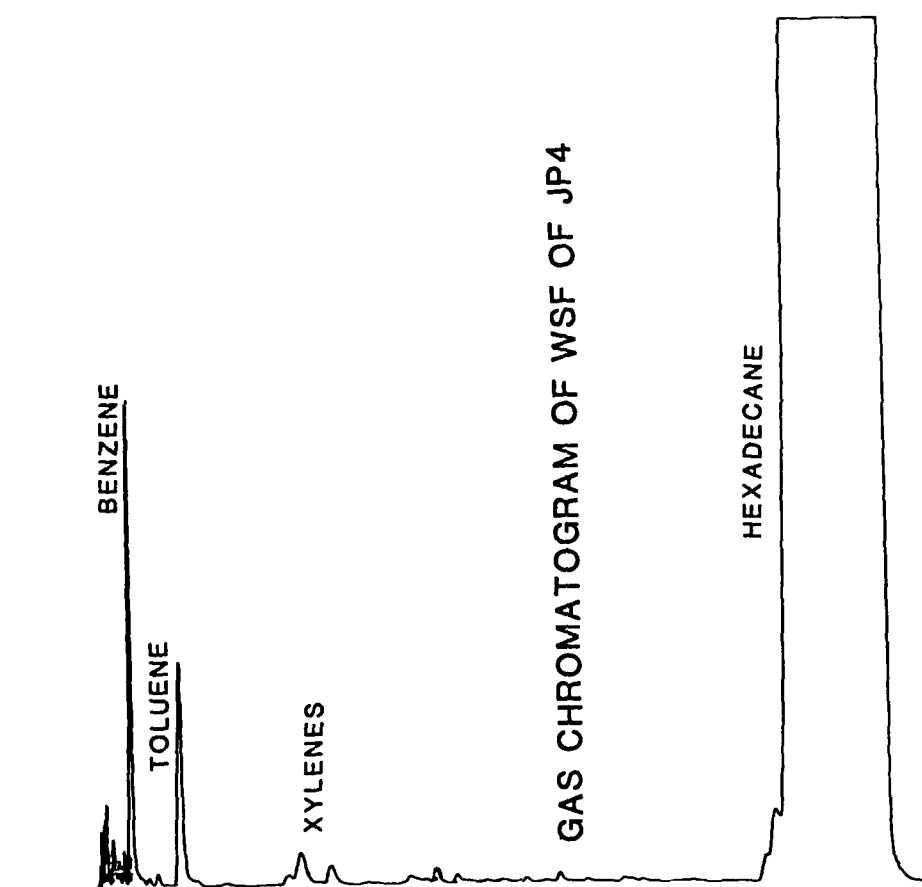


Figure 2: Representative gas chromatogram for WSF JP-4.

a. A Varian 1600 gas chromatograph with a 6 ft SE30 column and flame ionization detection is used to analyze for the jet fuel. The gas chromatograph is programmed to maintain 40°C for 5 minutes and then increase by 10°C min⁻¹ until a final temperature of 200°C is reached. The injector is set at 220°C and the detector at 225°C. A 6 ul sample is injected for each determination. A representative gas chromatogram is shown as Figure 2. As can be seen, toluene and benzene are the two largest components of the WSF of petroleum JP-4 jet fuel.

b. The toluene and benzene peaks are used to calculate the concentration of the WSF. A standard maximal WSF (MaxWSF) is made by mixing a 5% JP-4 and carbon filtered tap water sample for 3 hours at 25°C at a constant mixing speed. This mixture then settles for three hours and the WSF extracted is with hexadecane. This MaxWSF is the standard against which all other WSF concentrations are compared. The WSFs are usually referred to as %WSF, a per cent of the MaxWSF.

B. Toxicity of JP-4 Jet Fuel

1. To determine the sublethal effects of the WSF of JP-4 jet fuel, it was necessary to determine the lethal concentration of the material. Both static and dynamic bioassays were conducted. These tests were performed on juvenile bluegill sunfish (Lepomis macrochirus).

2. The static tests were run in 3 l glass aquaria with 10 fish in each exposure container. These bioassays were run

using JP-4 layered directly onto the water in the aquaria. (These were defined as being "neat" exposures as opposed to using the WSF of the JP-4.) An order of magnitude series of concentrations of the JP-4 was used for these bioassays. They were conducted as 96 hr tests with observations being made at 15 min, 1/2 hr, 1 hr, 6 hr and every 24 hours after that. The pH and dissolved oxygen in each tank were also determined on a regular basis.

3. The dynamic bioassays were conducted using an all glass and teflon Mount-Brungs dilutor system (Figure 3). The fractionator described above was used. Several changes were made to an existing dilutor system due to the volatility problems with the JP-4. All of the individual chambers had to be more specifically sized and lids fitted to avoid excess volatilization. There were also changes made to the internal delivery systems in the dilutor to eliminate excess volatilization. There were 10 fish used per concentration in this bioassay. Observations on lethality, pH and dissolved oxygen were made on the time basis discussed above for the static tests. This test was also conducted as a 96-hr test.

C. Ventilatory Rate Studies

1. Ventilatory studies were conducted on bluegills using the system developed at Virginia Tech. Fish were placed in small plexiglass chambers which had electrodes in the front and rear (Figure 4). Any movement of the fish was detected as a small voltage by these electrodes.

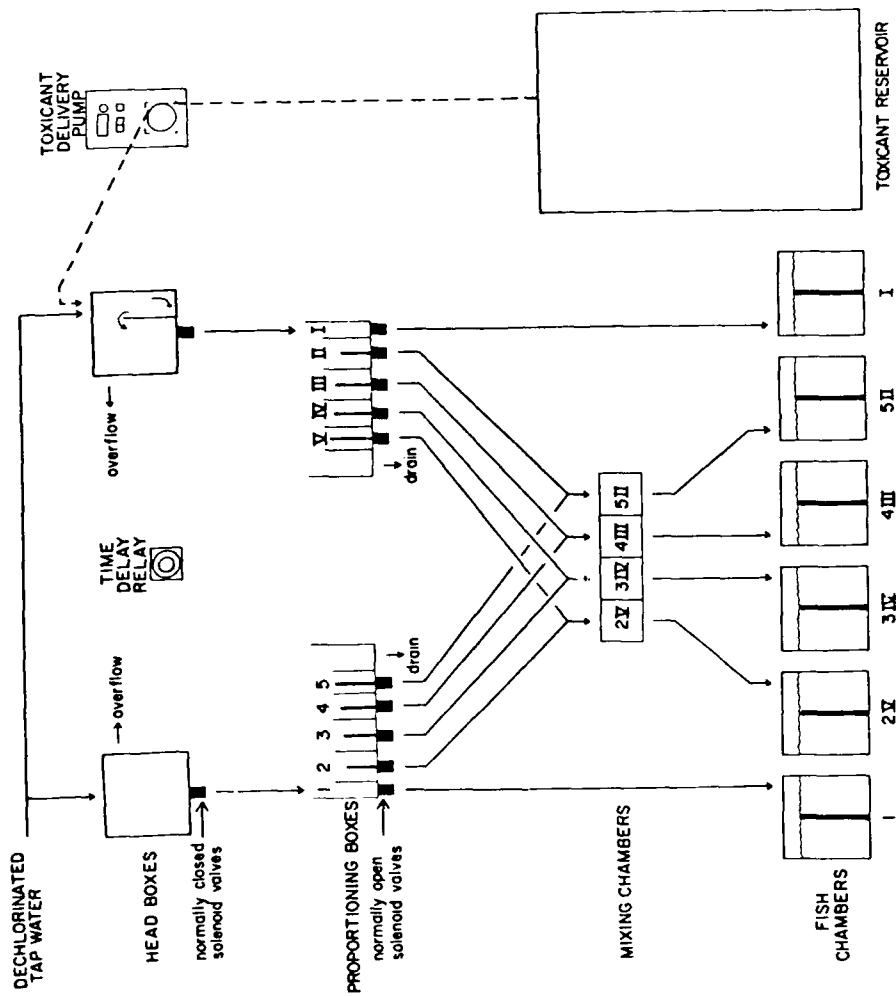


Figure 3: Schematic of Mount-Brungs dilutor system used to conduct dynamic bioassays.

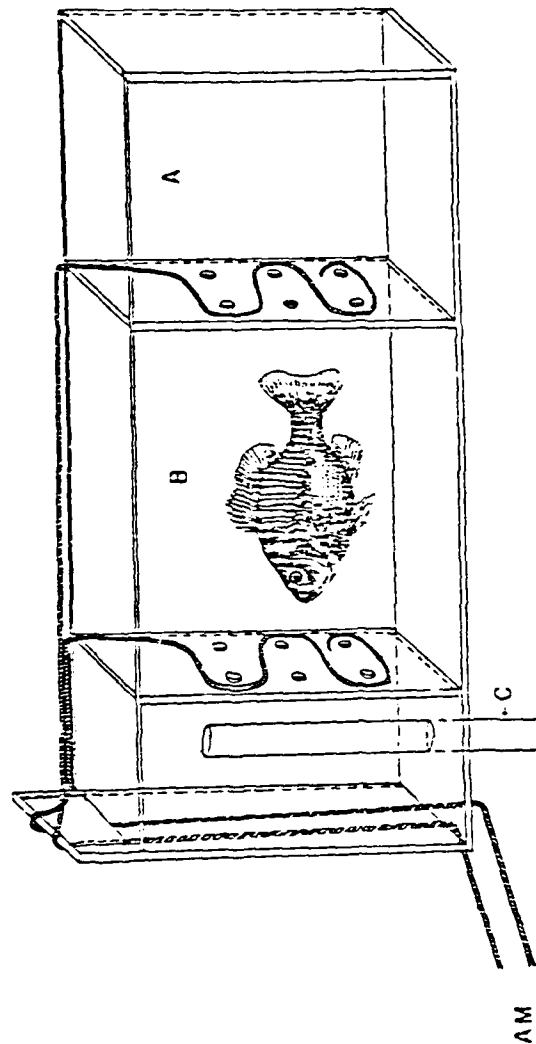


Figure 4: Schematic of fish tank used to hold fish for ventilatory rate study.

The chambers were sized such that the fish placed in them could not easily move around. The chambers were in a closed container which had constant light and was buffered from effects of any movement in the laboratory (Figure 5). This was done to eliminate as many extraneous stimuli as possible so that fish would only respond to changes in the concentration of the WSF JP-4. The fractionator previously described was used to produce WSF for this study.

2. Fish were allowed to acclimate in these chambers for 2 days before exposure to the WSF JP-4. During acclimation the fish were exposed only to carbon filtered tap water and background ventilatory rates were determined. These background rates were determined over at least a 24 hr period. Approximately half the fish were then exposed to the WSF JP-4 and data were collected for another 24 hrs.

D. Preference/Avoidance Studies

1. Preference avoidance studies were conducted using equipment modified from that previously used at Virginia Tech. Fish were given a side-by-side challenge of clean (carbon filtered tap water) and WSF JP-4 influenced water. Due to the requirement for high flow rates, a batch system, using two-55 gallon drums was used to produce the WSF JP-4 for this experiment. A 5% JP-4 solution was mixed for 6 to 12 hrs and then allowed to settle for 6 to 12 hrs. The concentration of the mixture was determined and the appropriate amount pumped into the exposure side of the preference/avoidance chamber.

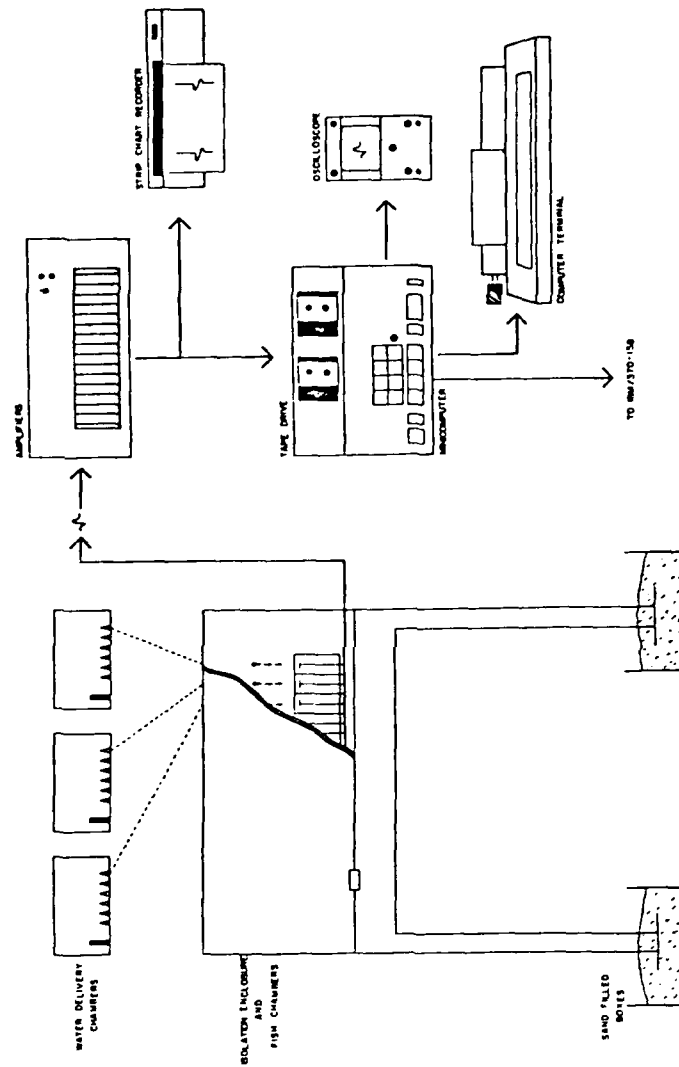


Figure 5: Schematic of apparatus used for fish ventilatory rate studies.

The exposure chamber was contained in an isolation box into which the clean and contaminated waters were pumped (Figure 6.) The behavior of the fish was observed with a closed circuit TV system. (This procedure is the same as in the fish ventilatory response study in that the fish were subjected to as little extraneous stimuli as possible.) There were three different observational techniques used to determine the behavior of the fish in the preference avoidance studies.

a. The fish were observed directly on the closed circuit TV system to determine their behavior. Five fish were placed in the exposure chamber for each study. During an observation period the positions of the fish were noted every 30 sec.

b. On alternate 30 second periods the number of movements of the fish were also noted. This was measured by dividing the TV screen into quadrants (Figure 7). Each time any fish crossed any quadrant line this was considered to be a movement.

c. Measurement of time the fish spent on the exposed side of the tank was also made using a computer that determined the actual position of the fish every 30 sec. All fish were then seen by the system as dark "pixels" on the light screen. The number of dark pixels for each quadrant were then totaled every 30 sec by the computer program.

2. Fish were placed in the sealed preference/avoidance

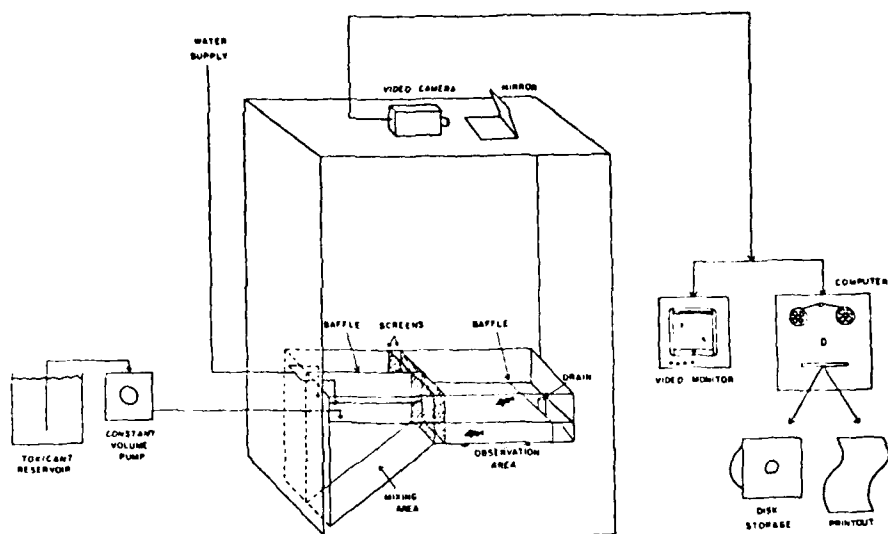


Figure 6: Schematic of apparatus used for preference/avoidance studies.

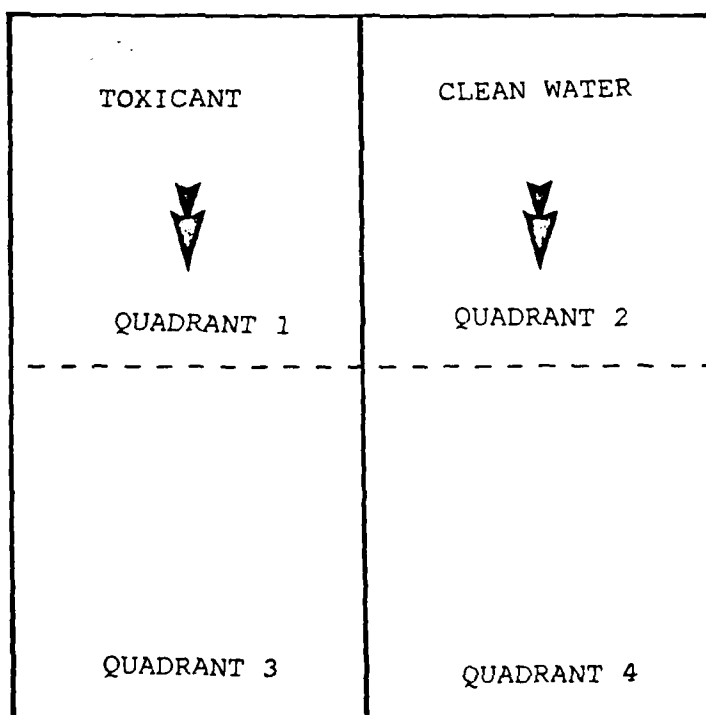


Figure 7: Schematic of preference/avoidance chamber showing the quadrants as used for fish movement determinations.

chamber and allowed to acclimate for one to two hrs. Observations were then made for the control (0% WSF) level. In the first study, three 10 min observations were made at each concentration. A sample was then taken from ports at the bottom of the exposure chamber. The concentration of WSF JP-4 was then increased to the next level. This exposure procedure is diagrammed as study 1 in Figure 8. This experiment was repeated eight times.

3. Two control runs were also conducted in which no WSF JP-4 was added; both sides of the exposure tank had only water. These studies were conducted over a six hr period to verify that there was no particular preference by the fish for any section of the exposure chamber.

4. Another study was conducted in which the fish were taken directly from no WSF JP-4 to a concentration approximately half of the 96-hr LC50 (study 2 in Figure 8). The purpose of this run was to determine if the fish habituated to the WSF JP-4. This study was conducted twice.

5. A last study was conducted in which the fish were exposed to an increase in concentration of the WSF JP-4 more rapidly than in study 2 (study 3 in Figure 8). Due to the requirement to maintain high flow rates, it was not possible to make as many observations at each level of concentration. In this study there was one 7 min observation of behavior at each concentration of WSF JP-4. This study was repeated 8 times.

PREFERENCE AVOIDANCE STUDIES

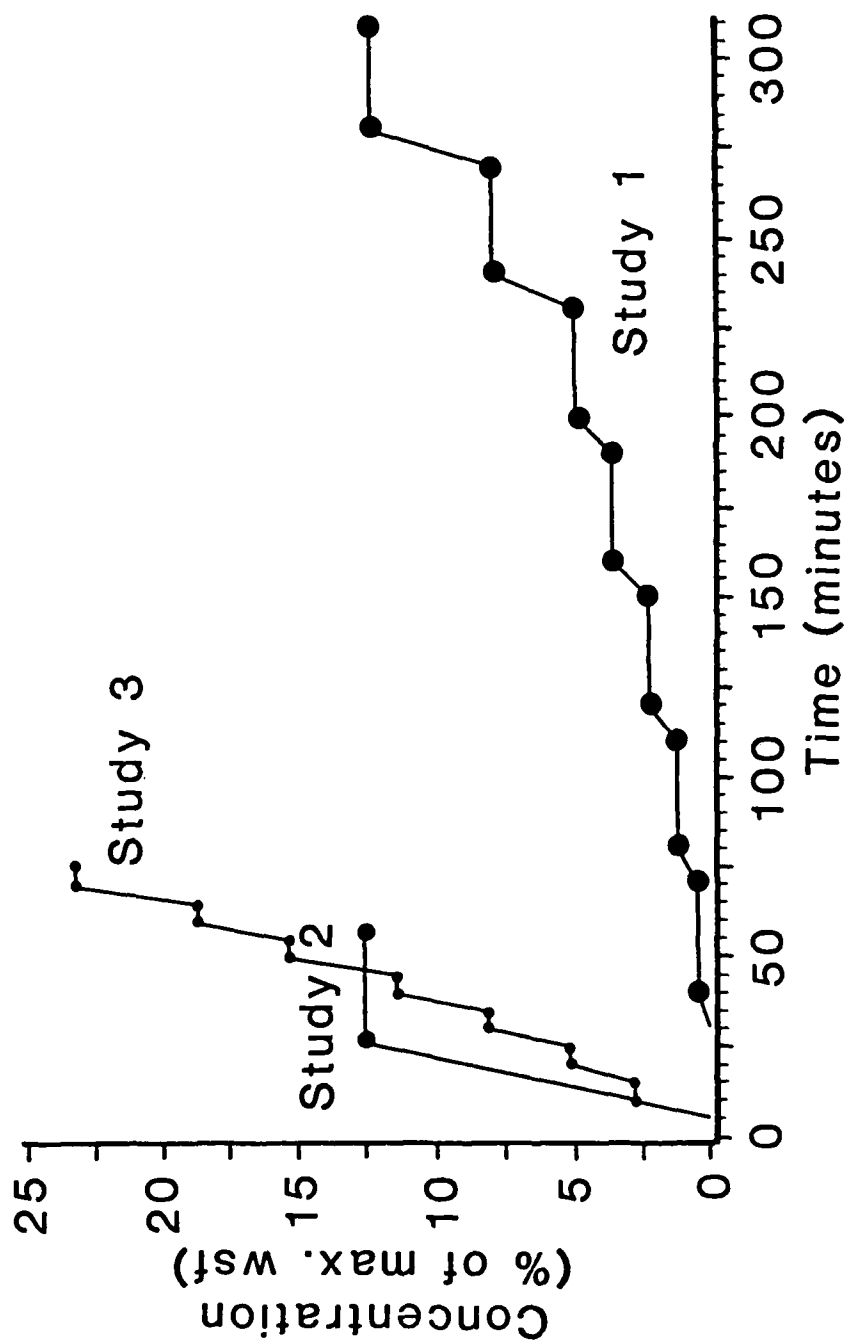


Figure 8: Methodology used for WSF exposures for preference/avoidance studies.

E. Blood Chemistry

1. Fish were exposed to two different concentrations of WSF JP-4 to evaluate the effect on blood chemistry parameters. The first exposure was 13 % WSF JP-4 for 96 hrs. This level was approximately half of the nominal 96-hr LC50. Other fish were exposed to approximately the 96-hr LC50, 26 % WSF, for 24 hrs to evaluate the effects of a lethal level. After this exposure period the fish were bled and several chemical and physical determinations were performed (discussed below).

2. The fish were bled after first being anesthetized with a five per cent benzocaine solution. The caudal peduncle was excised and blood removed from the dorsal aorta using 1 cc syringes. Two subsamples were taken for each fish blood sample. One subsample was treated with heparin and the other sample was not; this was for differing blood parameter analysis requirements. When possible some determinations were performed on the blood from each fish. In most cases blood from two or more fish was pooled into one sample in order to obtain sufficient volume to perform all of the desired analyses.

3. The blood parameters that were studied fell into two groups; whole blood and serum chemistry tests. The whole blood tests consisted of hematocrit, hemoglobin concentration and red blood cell (RBC) counts.

a. The hematocrit test, a percentage of blood cells in the whole blood sample, was performed using microhematocrit

centrifuge tubes. A tube was filled with blood directly from each sacrificed fish and the percent hematocrit was determined using a Clay-Adams Hematocrit Reader after the tubes were spun down in a Clay-Adams Hematocrit Centrifuge for 10 mins.

b. The hemoglobin determination was performed on the pooled samples of whole blood using the cyanomethemoglobin method.

c. The RBC counts were conducted after diluting the whole blood 1:200 with Dacie's solution and placing a small volume of diluted blood on a hemacytometer. Five ruled areas on the hemacytometer were counted and averaged. This average was multiplied by a factor that took into account conversion of ruled areas to mm^2 , depth of the hemacytometer, and pipette dilution. The final RBC determination was in units of cells mm^3^{-1} .

4. Blood serum tests were conducted for: a) the enzymes lactate dehydrogenase (LDH) and aspartate aminotransferase (GOT); b) inorganic substances calcium (Ca), chloride (Cl), magnesium (Mg), and inorganic phosphorous (Pi); c) albumin and total protein concentration; and d) glucose (determined through the use of glucose dehydrogenase (GDH)). These blood parameters were analyzed using a Gilford 3500 computer assisted blood chemistry analyzer and the applicable Gilford reagents.

5. There were several other techniques used to physiologically examine the condition of the fish.

a. Two different types of commercially available reagent strips for human urinalysis were used to examine the mucous on the fish. The first of these, Hemastix , evaluates the amount of hemolysis that has taken place in the fish blood. The hemolysis products of fish blood will be found in the mucous of the affected fish. The second mucous examination was conducted with Ketostix . These reagent strips evaluate the amount of ketone bodies that are present in the mucous of the fish. This would give a good indication if the fish were being starved. Under conditions of starvation and lack of sufficient glycogen reserves the fish would turn to fatty acid and protein metabolism. Ketone bodies would be a product of these types of metabolism and would be found in the mucous of the fish.

b. Osmoregulation in the fish was evaluated by determining the water content of muscle and liver tissue. Samples of tissues were taken from the fish and dried. The percent water content was calculated to determine if the exposure to WSF JP-4 had affected the water content of the fish tissue indicating an osmoregulatory problem.

c. ATP levels in the liver of the fish were also determined. The technique used was an enzymatic one in which ATP reacted with phosphoglycerate kinase and the reaction was observed with a spectrophotometer.

F. Fish Histology

1. The original research plan was to examine both liver and gill tissue from exposed fish. It was later decided to

also examine olfactory tissue to determine if the lack of consistent avoidance behavior might be due to altered tissue structure.

2. The fish were exposed to the same concentrations as in the blood chemistry studies discussed above. These were 13% and 25% WSF JP-4 for 96 hrs and 24 hrs, respectively. The tissues were removed from the fish immediately after they had been bled. The tissues were placed in a gluteraldehyde fixative and later into an osmium tetroxide fixative. After being fixed, the tissues were dehydrated with a series of increasing concentrations of alcohol:water dilutions ending with a 100% alcohol solution. After dehydration they were embedded in a Spur's embedding media. The embedded tissues were then shaped and sectioned on a microtome. Thick sections were made for use with a light microscope and thin sections were made for use with a electron microscope.

a. A Leitz Dialux microscope with an attached camera was used to examine the thick sections of tissue.

b. A JEOL-JEM 100C electron microscope was used to examine the thin sections of the tissue.

III. RESULTS AND DISCUSSION

A. Manufacture and Analysis of WSF JP-4

1. The fractionator efficiently generated a reliably uniform concentration of WSF JP-4. The only persistent long term problem experienced with the equipment, aside from

occasional tubing failures and the like, was the growth of a microorganism capable of biodegrading the components of the WSF JP-4 relatively quickly. We are currently attempting to identify this organism.

2. The analysis of the WSF JP-4 showed that the two primary components of the WSF were toluene and benzene. These were found to be present in the MaxWSF at 16.4 ppm benzene and 12.2 ppm toluene. These values agreed very well with those provided by AFWAL/POSF and AMRL/THE Wright-Patterson AFB OH. As the purpose of this research is to examine and compare the sublethal effects of a petroleum and a shale derived jet fuel a detailed discussion of the chemical composition of these materials is not within the scope of this research. Such information can be found in other USAF publications.

B. Toxicity of JP-4 Jet Fuel

1. The 96-hr LC50 for the "neat" (pure) JP-4 was 1.85 ppm in the static test. Although this is reported as a 96-hr result, due to the high volatility of the jet fuel components it is actually only a 24-hr result. The concentrations of the main JP-4 components, toluene and benzene, were significantly reduced within less than 24 hrs. This LC50 value is in close agreement with similar determinations on other fish as found in the literature.

2. There were problems in generating the dynamic bioassay data due to a lack of partial kills. In order to use the normal Probit analysis for bioassay calculations, it is

necessary to have at least two partial kills, as well as 100% survivals and 100% kills to generate reliable data. We got no more than one partial kill in any of our replications. Due to this problem, we used a Spearman-Kärber statistical analysis which does not depend on the presence of partial kills to generate an LC50. With this statistical technique, the calculated 96-hr LC50 was 26.2% MaxWSF.

3. In order to evaluate the shape of the toxicity curve, the data from all replicates of the dynamic bioassay were combined for a Probit analysis. The results of this are shown in Figure 9. Using this procedure a 96-hr LC50 of 29.2% WSF was generated. (These two values for the 96-hr LC50 are not significantly different.) The advantage of doing the Probit analysis on all the data even though it is not a normally accepted procedure is that it illustrates the steepness of the lethality curve that is generated. Ninety nine percent of the lethality occurs between 19.5 % WSF and 38.8% WSF.

4. The 24 hr, 48 hr and 72 hr LC50's were also determined using Probit analysis of all available data. These results are summarized in Table 1. It should be noted the four LC50's are not significantly different.

Table 1: LC50's for WSF JP-4 at 24, 48, 72 and 96-hrs.

24-hr LC50	= 25.5% WSF
48-hr LC50	= 26.4% WSF
72-hr LC50	= 28.7% WSF
96-hr LC50	= 26.2% WSF

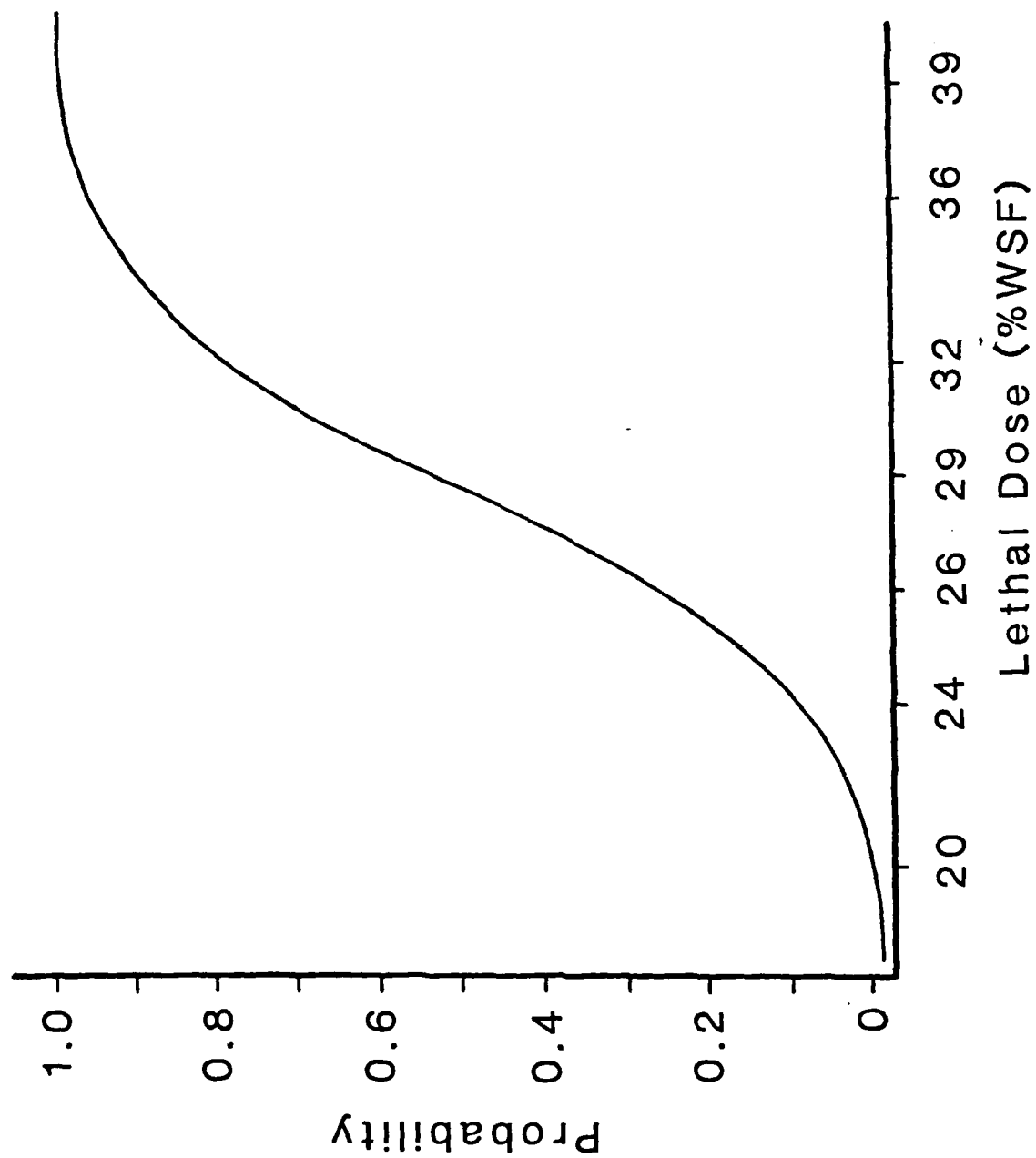


Figure 9: Probability curve for toxicity of WSF JP-4.

C. Ventilatory Study

1. When the ventilatory rates were compared to the concentration of the WSF JP-4 to which the fish were exposed there is a clear relationship ($p=0.0003$, $r^2=0.72$, Figure 10). This relationship is also seen if only the difference in the ventilatory rates before and after exposure are graphed vs. the concentration of WSF JP-4 ($p=0.003$, $r^2=0.71$, Figure 11).

2. In the original analysis, the fish were exposed to the WSF JP-4 for 24 hrs after the collection of background data. The statistical analysis was made by comparing the last 4 hrs of control data to the 4 hrs of exposed data after waiting 2 hrs for the WSF JP-4 to fully fill each exposure chamber. Due to some problems with the diurnal variation in fish ventilatory rates a second technique was also used. In this technique, the exposed fish were compared to control data that had been collected during the same time of the previous day to avoid this diurnal change (Figure 12).

3. Several statistical techniques were used to analyze the ventilatory data. Originally the exposed fish were viewed as a block for comparison before and after exposure and to the block of control fish. This was done using an analysis of variance (ANOVA) program. It was later determined that a nonparametric technique was more appropriate when the ventilatory data were determined not to be strictly normally distributed.

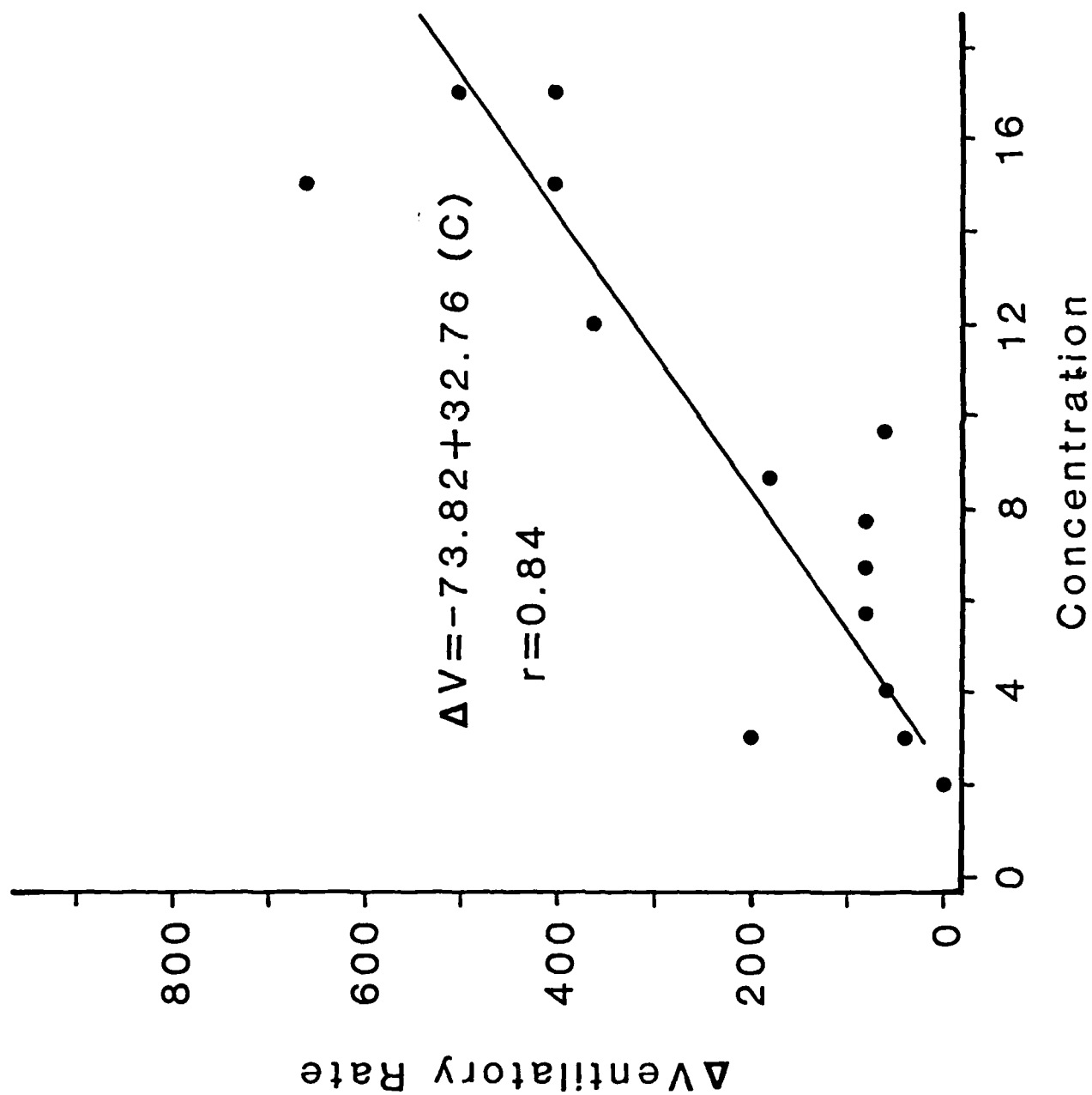


Figure 10: Graph of ventilatory rate vs concentration of WSE JP-4.

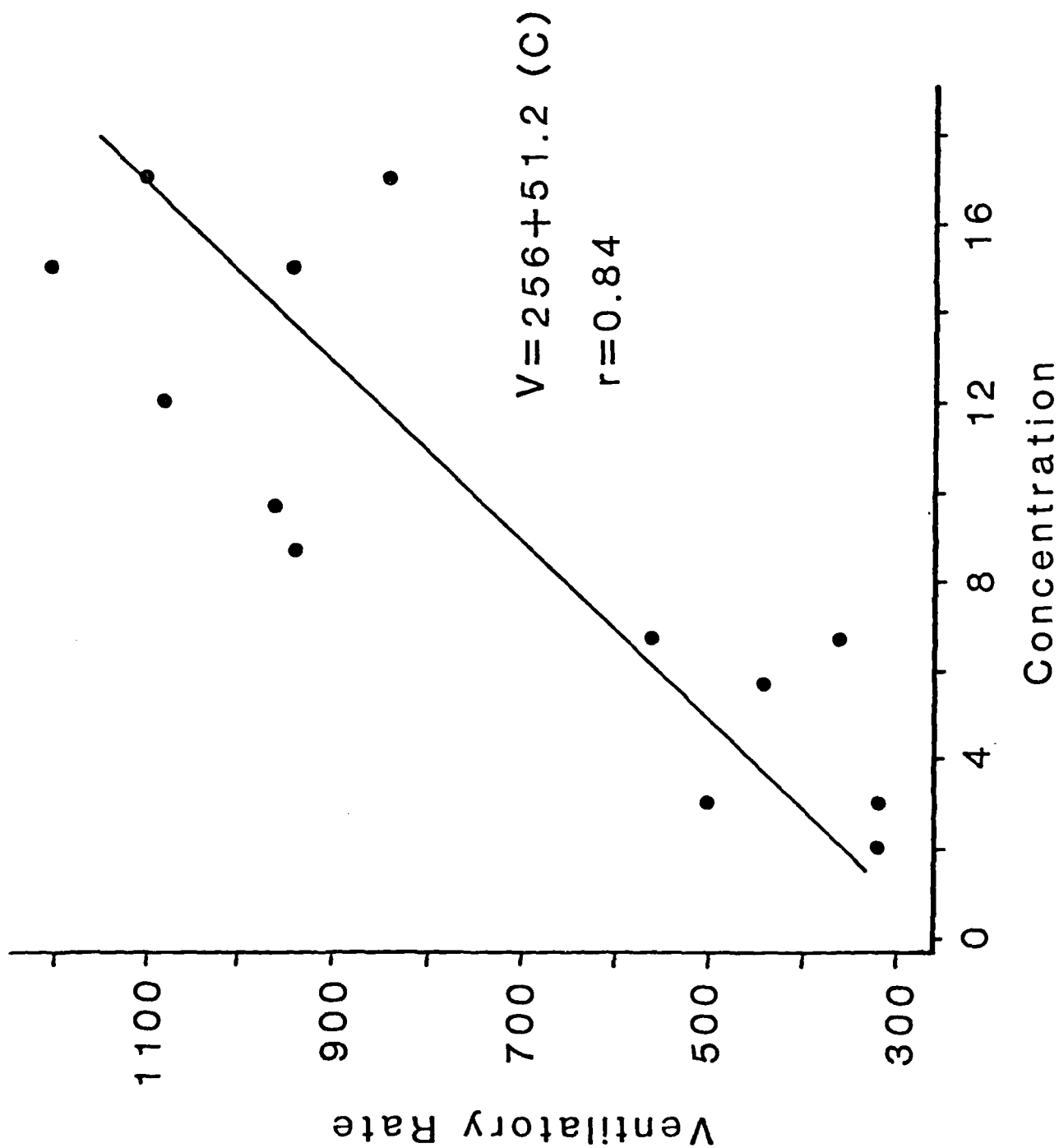


Figure 11: Graph of change in ventilatory rate from control vs concentration of WSF JP-4.

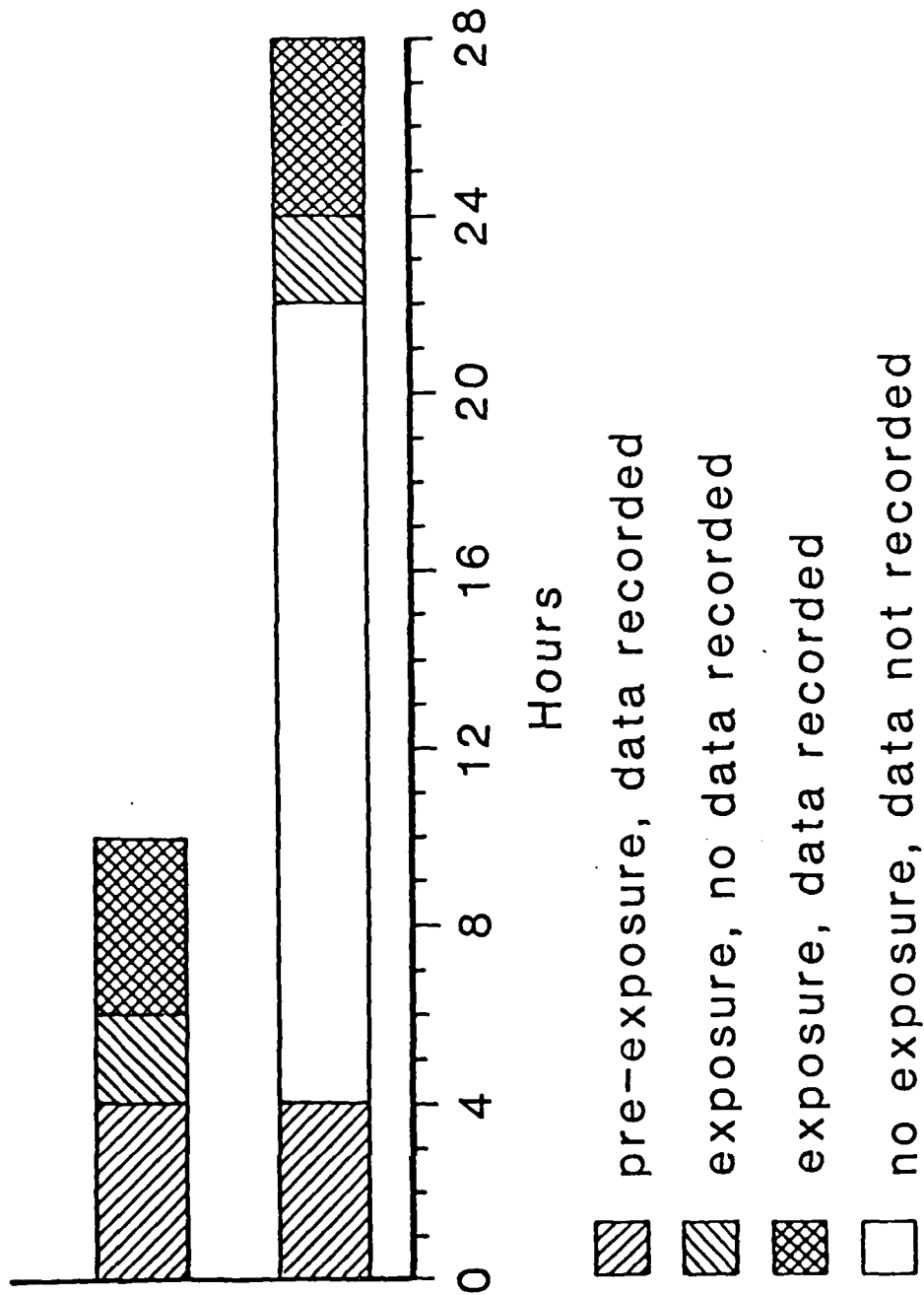


Figure 12: Methodology for collecting data from ventilatory rate study.

The statistical technique chosen was a paired rank sum procedure that compared the differences in ventilatory rate before and after exposure and then ranked the comparisons. This analysis was performed on all of the data. However, the 24 hr studies were more valid based on the data for the control fish. The results of the statistical analyses on the ventilatory data are in Table 2. Using a 0.05 level of confidence as the cutoff level the 5.1% WSF concentration was determined to be the level at which a "threshold" shift in ventilatory rate could be detected. This was approximately 20% of the nominal 96-hr LC50. However when compared to the Probit generated graph of lethal concentration values (Figure 9) this value is less than the 96-hr LC01 or the amount that could be expected to kill only 1% of the fish exposed to it for 96 hrs.

D. Preference/Avoidance Studies

1. There were two replicates in which the fish were not exposed to any WSF JP-4: this was the control for fish behavior over time. These studies were conducted over 5 hrs. As can be seen from Figure 13, there was no significant difference in the side that the fish chose over time. There was also no significant change in the number of movements over the time of these control runs.

2. In the first study, the fish were exposed to dilutions of the WSF JP-4 of up to 12% WSF (Figure 14). In this study the fish spent significantly different amounts of time on the exposed side of the chamber ($p=0.002$).

Concentration of Petroleum JP-4 (% maximum WSF)	Number of Fish	4 Hour Exposed vs 4 Hour Control (2 hour break)				4 Hour Exposed vs 4 Hour Control (24 hour comparison)			
		Exposed		Control		Exposed		Control	
		Vent.	Amp.	Vent.	Amp.	Vent.	Amp.	Vent.	Amp.
2.1	9	0.025	0.429	-	-	0.361	0.220	-	-
2.8	7	-	-	-	-	0.148	0.200	0.250	0.250
3.1	7	0.109	0.289	0.109	0.344	0.109	0.289	0.109	0.344
5.1	7	0.023	0.031	-	-	0.047	0.016	-	-
6.7	9	0.002	0.410	0.289	0.500	0.004	0.064	0.234	0.500
8.5	8	0.039	0.130	0.140	0.422	0.008	0.004	0.234	0.156
10	9	0.005	-	0.416	-	-	-	-	-
12	4	0.062	0.125	0.219	0.219	0.062	0.188	0.500	0.344
14	5	0.031	-	0.371	-	-	-	-	-
15	13	0.0007	0.001	0.013	0.027	0.0007	0.0007	0.016	0.289
17	8	0.004	0.008	0.027	0.38	0.004	0.004	0.004	0.422

Table 2: P-values for ventilatory rate studies for exposure to varying concentrations of WSF JP-4.

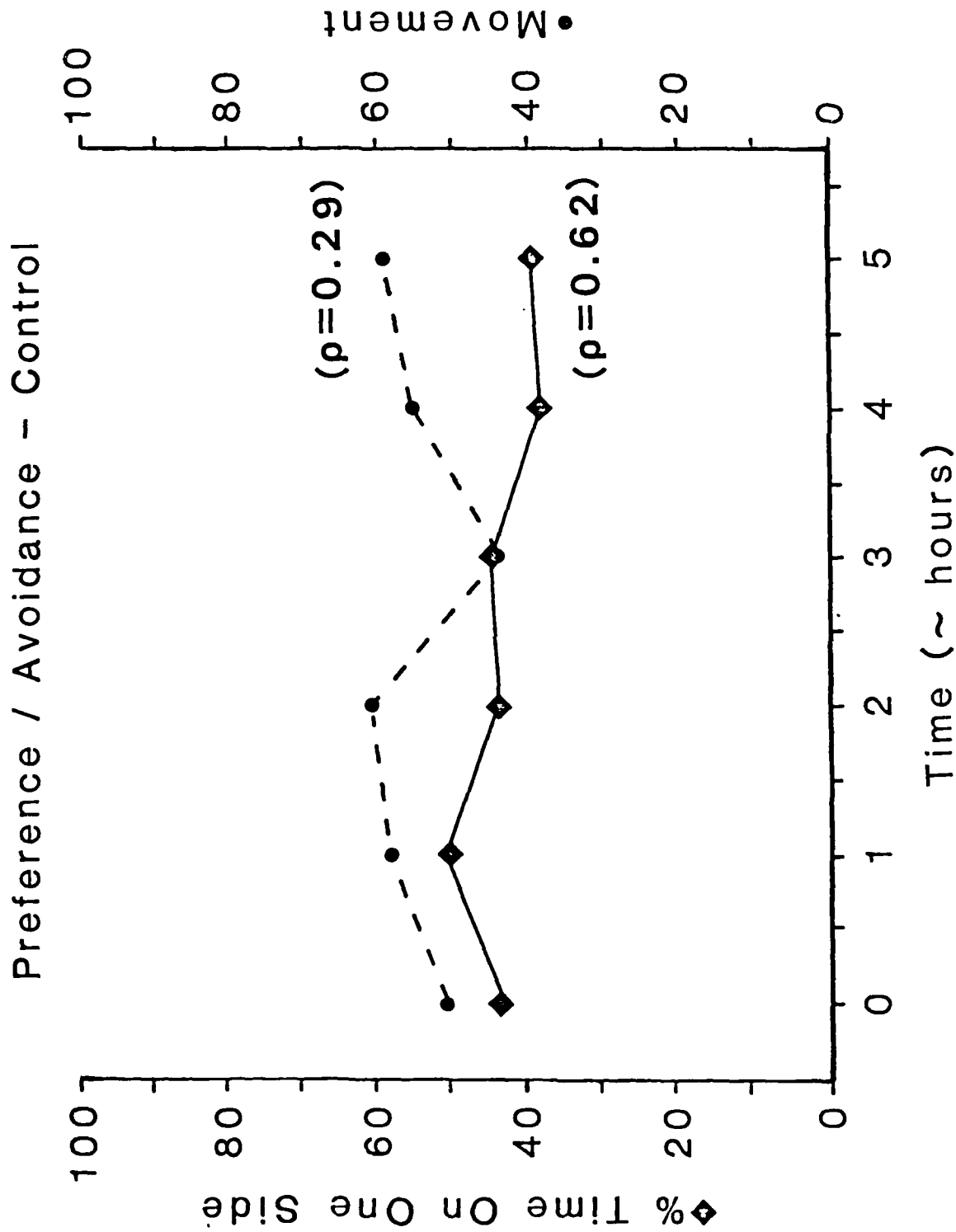


Figure 13: Graph of preference/avoidance behavior and fish movement over time with no WSF JP-4.

PREFERENCE AVOIDANCE - STUDY 1

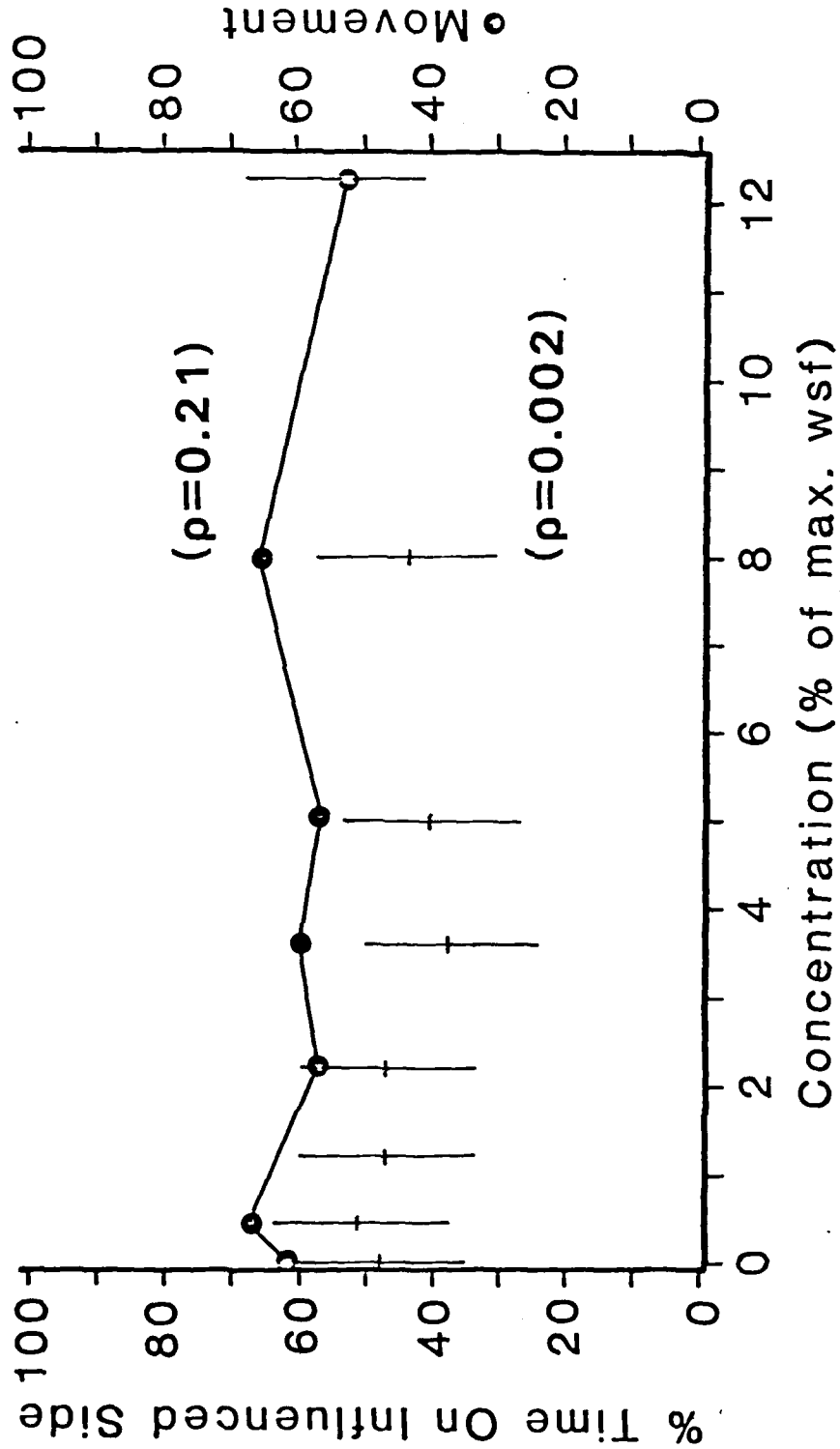


Figure 14: Graph of preference/avoidance behavior and fish movement for study 1.

When the different groups of exposures were analyzed using an LSD procedure it was determined that there was a definite trend indicating less time spent on the JP-4 influenced side when they were exposed to the median levels of WSF JP-4 (3.5% and 4.9% WSF). However, the fish show no difference in behavior at the highest concentration as compared to the controls. There are at least two possible explanations for this. The first is that the fish are fairly rapidly desensitized to the WSF JP-4 perhaps due to olfactory tissue damage. This will be evaluated by electronmicroscopic examination of the tissue of olfactory tissue from fish that have been exposed to WSF JP-4. Another possible explanation is that the fish become habituated to the WSF JP-4 as the level slowly increases in the preference/avoidance exposures. In order to test for this possibility another type of preference/avoidance study was conducted.

3. In this second test the fish were exposed to the control (0%) concentration and then to the highest level of exposure from study 1. If in study 1 fish acclimation determined behavior we hypothesized that they would actively avoid the sudden exposure to the high concentration of WSF JP-4. The fish showed no significant difference in avoidance or movement behavior between the control and high exposure ($p=0.12$ and 0.98 , respectively, Figure 15).

4. The third study was conducted with more rapidly increasing concentrations than study 1 in order to determine what the fish behavior would be when the exposure

PREFERENCE AVOIDANCE - STUDY 2

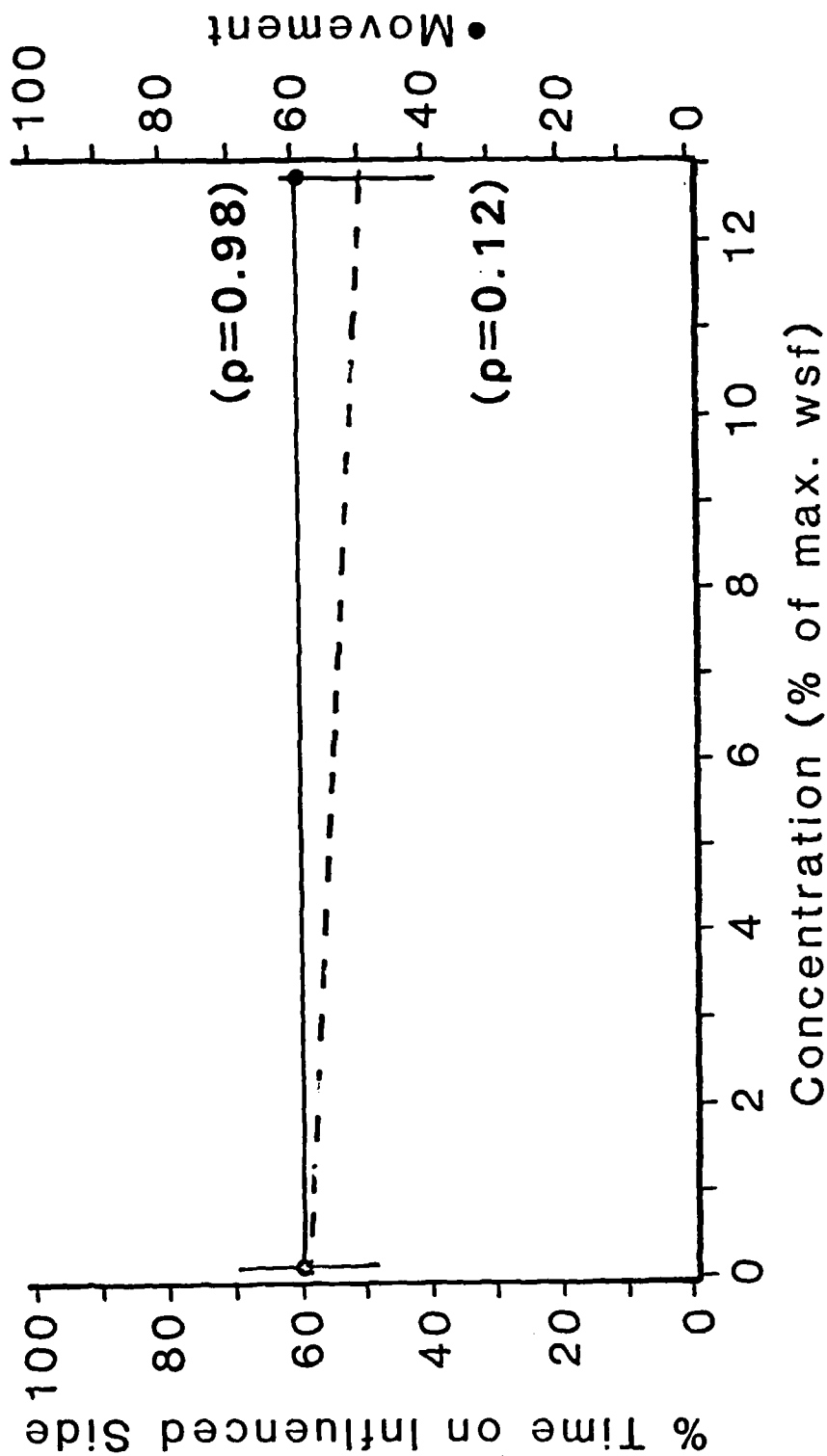


Figure 15: Graph of preference/avoidance behavior and fish movement for study 2.

level reached the 96-hr LC50. (In this study observation times were shortened due to the requirement for larger total quantities of WSF JP-4 then could be reasonably prepared.) There was no significant difference in either the avoidance or movement behavior in this study ($p=0.08$ and 0.058 , respectively, Figure 16). This may partially be due to the rapidly increased concentration and the high level of "noise" in the fish behavior due to the fewer number of observations. It should be noted that these p -values are not far from being significant at 0.05 and that the data points for avoidance behavior at the median levels of concentration, 3.7% and 5.2% WSF JP-4, are distinctly lower than for control and high, 23.3% WSF, concentration. Study 3 would therefore seem to reflect the results that were seen in studies 1 and 2 although not as significantly.

E. Blood Chemistry/Physiology

1. Fish were initially exposed to 13% WSF JP-4, half of the nominal 96-hr LC50. This concentration was actually the 96-hr LC04 based on the Probit curve (Figure 9). Although this exposure was for 96 hrs it would not be likely that there would be many physiological changes in the fish at an LC04 concentration. A second study was conducted with an exposure concentration which approximated the 96-hr LC50, 26% WSF JP-4. This study would indicate the changes that could be expected in the fish just before the concentration became lethal. This second exposure lasted for 24 hrs due to the anticipated high mortality of the exposed fish.

PREFERENCE AVOIDANCE - STUDY 3

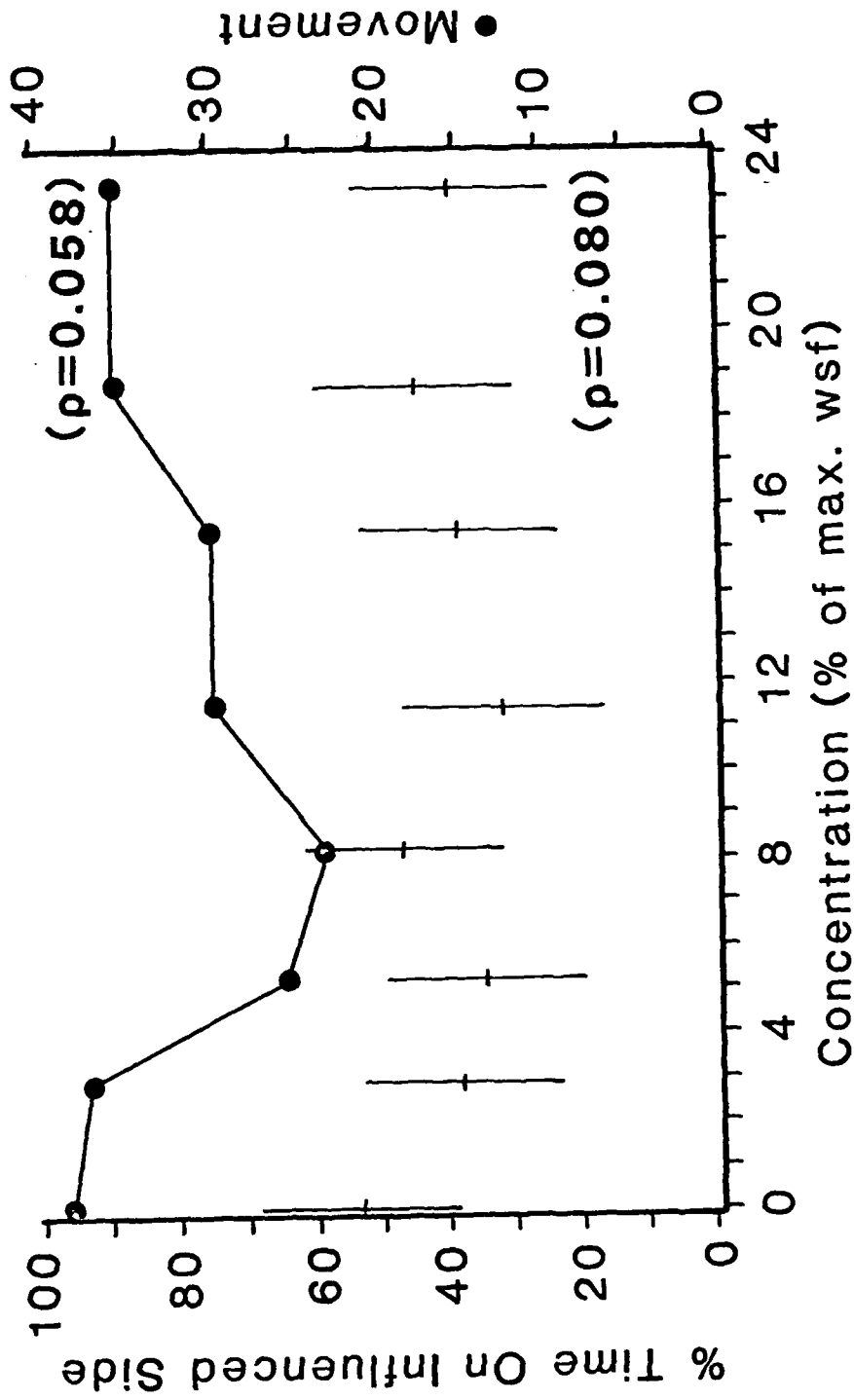


Figure 16: Graph of preference/avoidance behavior and fish movement for study3.

2. At the higher concentration there was approximately a 50% kill. This was satisfying since the lethality determinations were based on experiments with juvenile fish and these studies were performed on adult fish. Although there was no narcosis of the fish seen in the preference/avoidance studies there did seem to be such an effect in this study just before the fish expired.

3. The results for these two studies are given in Tables 3 and 4 respectively.

4. Looking first at the "whole body" parameters, hemolysis (Hematostix) ketosis (Ketostix) and percent water content of the flesh, the lower WSF JP-4 exposure resulted in no significant differences between the control and the exposed fish. At the higher concentration there was a significantly greater percent water content in the muscle of exposed fish. This would suggest osmoregulatory problems in the fish at this almost lethal concentration.

5. In the three whole blood component measurements, hemoglobin, hematocrit and red blood cell counts, there were no significant differences observed in the fish exposed to the lower WSF JP-4 concentration. At the higher concentration there was a significant decrease in the hemoglobin concentration, in percent hematocrit and number of red blood cells. The most likely explanation of this is that there is a blood dilution effect due to the osmoregulatory problem mentioned above.

Table 3: Physiological and blood parameters measured from
bluegill sunfish exposed to 13% WSF JP-4 for 96 hr.

Parameter	Control	Exposed	p-value
Ketostix (mucous)	0	0.3±0.83	0.39
Hematostix (mucous)	4.14±3.4	3.93±2.96	0.8
Water content-flesh (%)	74.9±0.6	75.3±0.5	0.7
Hemoglobin (g/100ml)	7.99±0.73	8.03±0.78	0.9
Hematocrit (%)	46.8±4.8	46.0±5.4	0.6
Red blood cells (x10 ⁶ /mm ³)	1.44±0.43	1.69±0.47	0.5
Calcium (mg/dl)	44.63±17.51	43.73±17.52	0.9
Chloride (mg/dl)	139±114	108±121	0.5
Magnesium (mg/dl)	5.44±2.99	4.87±0.70	0.6
Phosphorous (mg/dl)	33.46±15.31	38.00±16.24	0.6
Total protein (g/dl)	7.48±0.93	8.15±0.91	0.07
Albumin (g/dl)	2.55±0.57	2.62±0.40	0.7
Lactate dehydrogenase (ug/l)	4942±1186	5276±1658	0.6
Aspartate aminotransferase (ug/l)	500±109	620±147	0.1
Glucose (mg/dl)	66.09±8.29	101.63±60.93	0.08
Water content-liver (%)	70.0±0.8	71.3±1.8	0.023
Adenosine triphosphate (liver)	3.86±0.80	2.90±0.63	0.01

Table 4: Physiological and blood parameters measured from
bluegill sunfish exposed to 25% WSF JP-4 for 24 hr.

Parameter	Control	Exposed	p-value
Ketostix (mucous)	0	0	
Hematostix (mucous)	7.00±0.76	6.32±1.72	0.2
Water content-flesh (%)	76.0±0.4	77.0±0.8	0.0006
Hemoglobin (g/100ml)	6.58±0.74	4.52±1.09	0.0007
Hematocrit (%)	39.9±4.4	34.5±4.4	0.01
Red blood cells (x10 ⁶ /mm ³)	155.5±13.1	87.4±26.6	0.0001
Calcium (mg/dl)	30.2±2.4	28.3±6.2	0.3
Chloride (mg/dl)	93.8±8.5	73.9±10.5	0.0001
Magnesium (mg/dl)	4.30±0.86	3.66±1.52	0.4
Phosphorus (mg/dl)	24.7±5.6	29.4±3.4	0.03
Total protein (g/dl)	6.25±0.82	5.74±0.86	0.2
Albumin (g/dl)	1.99±0.27	1.88±0.54	0.6
Lactate dehydrogenase (ug/l)	3571±1173	4860±2148	0.6
Aspartate aminotransferase(ug/l)	511±181	1134±474	0.0008
Glucose (mg/dl)	80.0±26.4	412±189	0.0001
Water content-liver (%)	69.7±1.2	77.4±3.9	0.0001
Adenosine triphosphate (liver)	3.75±0.95	1.30±0.83	0.0001

6. There was no significant change in the four serum ions that were measured in the fish exposed to the lower concentration. At the higher concentration both blood chloride and phosphorous ion concentrations were significantly lower; calcium and magnesium were not significantly changed. No explanation is proposed for this effect at this time.

7. There were no significant shifts in the serum enzymes LDH and aminotransferase for the fish exposed to the lower WSF concentration. Neither were there any changes in the total protein or albumin levels. Glucose was the only serum parameter which came close to being significantly elevated ($p=0.058$). At the higher concentration there was no shift in the level of serum LDH, but the level of aminotransferase was significantly elevated. This is the first change that can not be logically attributed to the suspected osmoregulatory problem in the fish exposed to the higher concentration. It is hypothesized that this was related to metabolism of accumulated JP-4 components. There was no difference in either the levels of total protein or albumin. There was a pronounced increase in the level of glucose in the blood of the exposed fish. This may be due to the fish mobilizing their stores of glycogen.

7. There were significant differences at both concentrations for effects on the liver. Both exposure levels resulted in increased amounts of water content of the liver. This could be the result of osmoregulatory problems

and perhaps a result of changes in liver tissue because of the sequestering of metabolites. There was a significant decrease in the amount of ATP present in the liver of fish exposed to both concentrations.

F. Histology

1. Due to the inherent problems in reproduction the actual photographs and electronmicrographs that were taken of the various tissues will not be reproduced in this interim report. They are available on request and will be published in the final report. The significant results observed will be reported here. Only the tissues from the lower WSF concentration have been evaluated at all at this time.

2. There were no obvious effects observed in the gills of the exposed fish as compared to the gills of the control fish when observed by either light or electron microscopy.

3. Under light microscopy the livers of the fish exposed to the lower concentration showed globular type bodies that stained differentially from the majority of other tissue. These bodies may be sequestered metabolic byproducts. Similiar observations have been reported in the literature. There seemed to be a decrease in the amount of glycogen stored in the liver of the fish exposed to the WSF JP-4. This correlates well with the perceived increases in serum glucose. There did not seem to be any other significant changes in the tissue when observed by either light or electron microscopy.

4. There were no definitive observed changes in the olfactory tissue. However, there did seem to be a possible effect seen on the number and condition of the cilia present. This type of effect has been reported in the literature, but more tissue samples need to be studied before a definitive statement to this effect can be made.

V. CONCLUSIONS

A. The dynamic 96-hr LC50 for the WSF of petroleum JP-4 jet fuel was 26% of the maximum soluble fraction (MaxWSF). The toxicity curve was very steep with 99% of the toxicity (LC01 to LC99) occurring between 19.5% and 38.8% of the MaxWSF. The 24-hr, 48-hr, 72-hr and 96-hr LC50's were not significantly different for WSF JP-4.

B. Increased ventilation rate in the exposed bluegill sunfish was detected at a sublethal concentration, 5% WSF JP-4. This was equivalent to 20% of the nominal 96-hr LC50 and less than the 96-hr LC01. Biomonitoring of the ventilatory rate of the bluegill may be an effective method for detecting sublethal amounts of the WSF of JP-4 jet fuel.

C. A second biomonitoring procedure, preference/avoidance behavior, proved to be a much less sensitive method of detecting sublethal levels of WSF JP-4. In one study there was a significant increase in avoidance behavior at concentrations similar to those that caused a change in ventilatory behavior. However this avoidance behavior was

not seen at higher concentrations. There was no indication of narcosis or anesthetization of the fish at any of the WSF concentrations to which the fish were exposed for the preference/avoidance studies. for the periods of time involved in the studies. In another study with higher concentrations of the WSF JP-4 there was no pronounced change in preference/avoidance behavior. This would indicate that the preference/avoidance behavior technique is not reliable for detecting the presence of jet fuel in the water. It is hypothesized that this is due to damage to the olfactory tissue of the fish.

D. There were few significant shifts in the physiological and blood parameters measured on fish exposed to a WSF concentration that was half of the nominal 96-hr LC50. The changes that were observed could be partially correlated with changes in the gill and liver histology. There were several changes in the physiological measurements for fish exposed to the higher concentration. This concentration was essentially the 96-hr LC50 and therefore the exhibited effects are those that just precede death in the fish. This would indicate that blood parameter analyses are not effective means for determining sublethal toxicity to bluegills resulting from exposure to WSF JP-4, at least after short term exposures. Even then the only really significant changes may be attributed to osmoregulatory problems. Since the gill tissue from the fish exposed to the higher concentration has not been examined yet, the

exact cause of this problem can not be stated at this time.

PART III

I. Introduction

This third part of the report will discuss the results of the research into the effects of the WSF JP-4 on invertebrate organisms. An abstract of this research can be found in Part I.

II. Materials and Methods

A. Equipment modifications

1. Invertebrates-- Tests of volatile materials with invertebrates pose special problems. Although rapid replacement of test material is necessary to maintain constant exposure concentrations, many invertebrates do not tolerate the turbulence associated with replacement. The Mount and Brungs dilutor (Figure 3) was modified to accomodate aquatic invertebrates by reducing test volumes and minimizing turbulence of the flow. One 225 ml aliquot of 6 serially diluted test concentrations were delivered to 1 L glass beakers every 20 min. Material in these central beakers was then siphoned into 3 replicate polyethylene test containers (102 mm diameter and 76 mm height) containing 325 ml test media. The inside diameter of the siphon tubes was 2 mm thus slowing the flow of test material into the test chambers and minimizing turbulence. A replacement volume of 325 ml was delivered to each test vessel every 1.5 hr.

2. Microbial Communities-- Definitive tests monitoring the chronic effects of WSF on microbial communities require

larger test chambers, higher continuous flow rates, and an additional headbox to accommodate the "epicenters" or species source pool. A system has been constructed for these tests (Figure 17).

B. Acute and Chronic Toxicity Tests With Invertebrates

1. Acute Tests-- Conditions for exposing the 3 invertebrates and microbial communities to the WSF of JP-4 jet fuel are summarized in Table 5. All tests were conducted on the modified Mount and Brungs dilutor. Light was provided by Durotest Optima fluorescent bulbs at an intensity of 20 ft-c at the air-water interface and a photoperiod of 16 hr light and 8 hr dark. Diluent water was carbon dechlorinated Blacksburg municipal tap water (hardness=60 mg/L). Acute toxicity tests monitored lethality at the end of a 48 hr exposure. Where possible, acute toxicity test data were analyzed using probit analysis to calculate LC50s. When a LC50 could not be calculated the percent survival in the highest concentration obtained was reported.

2. Chronic Tests-- Conditions for chronic tests were similar to those for acute tests and are also summarized in Table 5. Chronic toxicity tests monitored survival, growth, and/or reproduction for longer, variable periods of time. The chronic effects of WSF JP-4 on the survival of the invertebrates were evaluated using contingency table analysis to detect significant differences and probit analysis to examine dose response relationships.

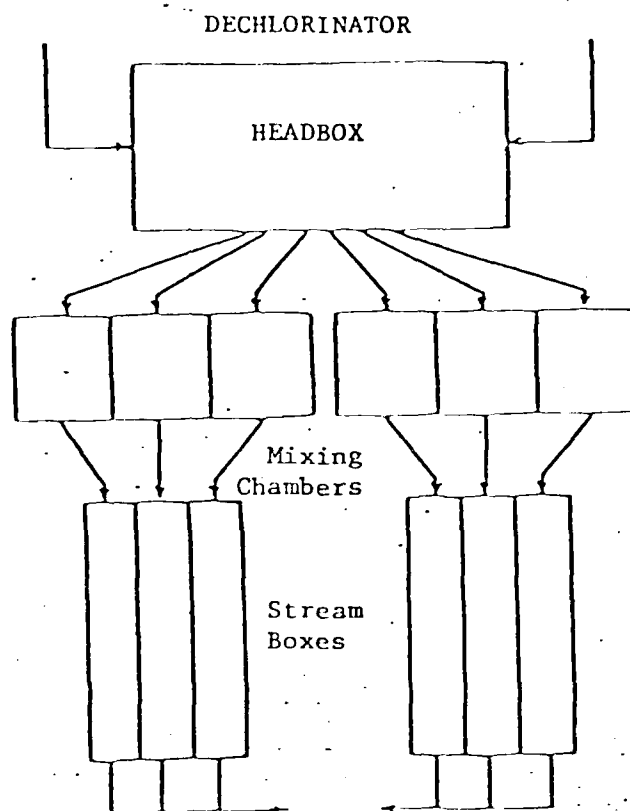


Figure 17: Schematic of a flow-through test system to measure colonization. Epicenter are placed in the head box, water and test compound mix and then enter the test troughs containing uncolonized PF islands.

Table 5: Conditions for acute and chronic tests exposing aquatic invertebrates and microbial communities to petroleum jet fuel WSF.

Organism Test Type*	Temperature (C)	Substrate or Food**	Length of Test (days)	Organism Size or Age	Response Measured	
<hr/>						
<u>Aelosoma</u>						
Acute	D	FT	25	450 mg/L TC	2	Small Death Reproduction
Chronic	D	FT	25	450 mg/L TC	7	
<u>Daphnia</u>						
Acute	D	FT, S	20	None	2	Death Reproduction
Chronic	P	FT	20	2.5 mg/L A	14	
<u>Paratanytarsus</u>						
Acute	D	FT	26	Sand	2	3rd Instar Egg Death Survival to adult
Chronic	P	FT	21	15 mg/L TC	28	
<u>Microbial Communities</u>						
Acute	D	FT	24	None	2	Mature Barren Species elimination Colonization
Chronic	P	FT	24	None	7	

* D=definitive test

P=preliminary test

FT=flow-through exposure

S=static exposure with replacement

**TC=Blended trout chow

A=Algae, Chlamydomonas reinhardtii

Effects on growth and reproduction were evaluated using one-way ANOVA and Duncan's multiple range test. The relationship between the WSF JP-4 concentration and the magnitude of the response was examined with ordinary least squares regression analysis. Where there was a significant relationship between dose and response an EC20, the concentration necessary to produce a 20% impairment in response relative to the mean control value, was estimated from the regression line by means of inverse prediction. The EC20 was chosen as the index of chronic effects wherever possible because, unlike Maximum Allowable Toxic Concentrations (MATC), No Observable Effect Concentrations (NOEC), and Lowest Observable Effect Concentrations (LOEC), the absolute value should not be dependent on sample size or other design factors. More powerful designs will narrow confidence limits around an EC20, not affect its size.

C. Acute and Chronic Toxicity Tests With Microbial Communities

1. Acute Tests-- Polyurethane foam substrates (PF substrates--3.75 x 5.0 x 3.0 cm) were suspended in a nearby pond and allowed to colonized until a relatively constant number of protozoan species was obtained. (Previous research has indicated that an equilibrium number of 40 to 60 species is reached within 10 to 14 d). These colonized PF substrates were retrieved and transported to the laboratory. Acute tests exposed colonized PF substrates to WSF JP-4 in the modified Mount and Brungs dilutor. The

number of protozoan species remaining on the PF substrates after a 48 hr exposure to the WSF was determined. Triplicate substrates were exposed to each concentration. However, to facilitate the time consuming process of species enumeration replicates were staggered over 3 d. The number of species remaining on the substrates after 48 hr were compared with one way analysis of variance.

2. Chronic Tests-- A preliminary chronic test monitoring the colonization of barren substrates over time in WSF JP-4 was also conducted. The modified Mount and Brungs dilutor was used for this test. A colonized sponge was placed in the central 1 L beaker and a barren PF substrate was placed in each of 4 replicate test chambers. Test media passed through the central beaker and over the species source pool or "epicenter" then was siphoned into the test vessels containing the barren substrates. These PF substrates were sampled through time. One substrate from each concentration was examined on days 1, 2, 4, and 7. The size of the test system precluded replication in this preliminary test. Colonization curves describing the accrual of species on the barren substrates over time according to the MacArthur-Wilson model of noninteractive island colonization were fitted and compared. Estimates of colonization rate and equilibrium species number were compared between concentrations.

III. Results and Discussion

A. Chemistry

1. Biodegradation-- The flow-through system for invertebrates maintained constant concentrations of WSF for periods up to 1 week with coefficients of variation <30 %. However, in longer tests there were problems with toxicant loss over time, probably due to increasing populations of bacteria which degraded jet fuel components. This problem is more fully described in the results for the Paratanytarsus tests, reported below. Similar biodegradation could be expected in the field and would certainly reduce the severity of any deleterious effects on aquatic systems from jet fuel exposure. Specific fate studies could document this loss. However, the constantly declining concentrations make toxicity test data difficult to compare and interpret. We are experimenting with several methods to discourage or control bacterial growth during the course of an experiment in order to maintain constant exposure concentrations over the course of a chronic test.

B. Invertebrate Toxicity Tests

1. Aeolosoma

a. Acute Tests-- Acute exposures to concentrations up to 8.9 % WSF JP-4 were not lethal to A. headleyi. Exposure to 20.6 % WSF JP-4, the highest concentration attained, was lethal to only 17 % of the organisms. Thus no LC50 can be determined for this test.

b. Chronic Tests-- Chronic exposures to WSF JP-4 resulted in significant decreases in the number of worms

present after 7 days ($p=0.0006$, Figure 18). The NOEC was 1.2 % and the LOEC was 8.9 % WSF. There was a significant relationship between WSF concentration and the number of worms at the end of the test ($p=0.0001$, $r^2=0.73$). The relationship was of the form:

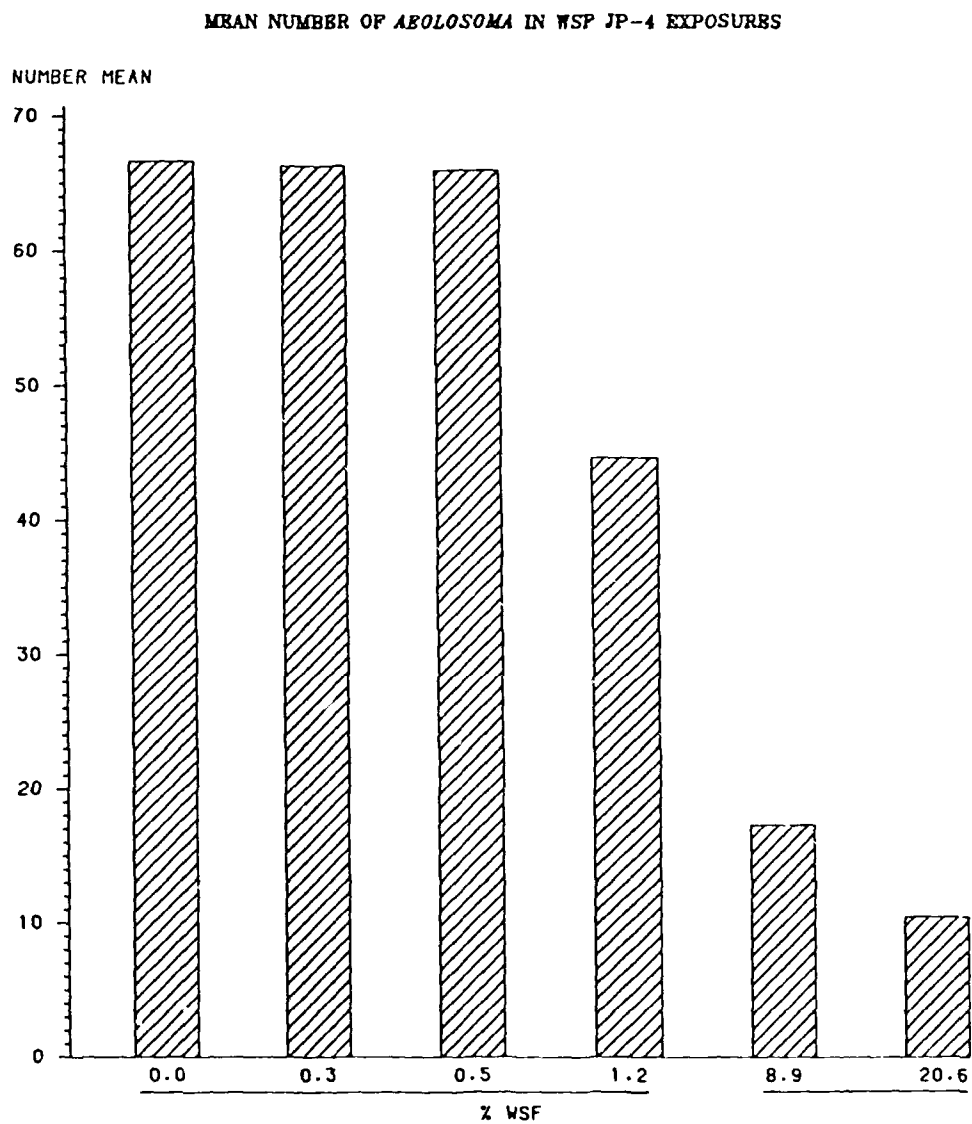
$$Y=4.07-0.1033 X$$

and there was no significant lack of fit ($p=0.2544$). The EC20 estimated from this relationship was 7.0 % WSF (1.3 to 13.3).

2. Daphnia

a. Acute Tests-- Two acute toxicity tests were conducted exposing D. pulex to the WSF of JP-4 jet fuel. The first test was static with renewal of the test solutions every 24 hr. There was 73 % survival in the highest concentration attained. In this test the undiluted WSF was 33.7 % MaxWSF at set up and renewal and declined to 17.2 % MaxWSF after 24 hr, averaging 25.5 %. In the flowthrough test the highest concentration attained was 13.3 % and there was 100 % survival in this concentration.

b. Chronic Tests-- A chronic exposure of D. pulex to WSF JP-4 has been conducted. There was 93 % survival at the highest concentration attained (11.7 % WSF JP-4) and animals in this concentration were reproducing well, producing an average of 40.4 young per female in 14 d. However, the control organisms in this test produced only 7.3 young per female in 14 days as compared to the 40 young per female in 21 days



MEANS CONNECTED BY THE SAME LINE WERE NOT SIGNIFICANTLY DIFFERENT ($P=0.05$)

Figure 18: Effects of WSP JP-4 exposure on reproduction of Aeolosoma headleyi.

considered the minimum for healthy control organisms (Figure 19). This suggests unfavorable conditions in the control vessels and we suspect that an insufficient food supply was responsible for low reproductive rates. In fact, reproduction increased with exposure concentration of WSF JP-4. It is possible that daphnids were eating bacteria encouraged by the presence of the jet fuel. The increase in high quality bacterial food resulted would then result in increases in reproduction.

3. Paratanytarsus

a. Acute Test-- The 48 hr LC50 for third instar P. parthenogenetica exposed in dechlorinated tap water was 2.2 % WSF (95% fiducial limits 1.6-2.9). The slope of the log dose-probit response line was 1.85 (95% confidence interval 1.59-2.11).

b. Chronic Test-- Preliminary analyses of chronic test results for P. parthenogenetica show a one week LC50 of 2.8 % WSF (2.2-3.7). The slope of the log dose-probit response line was 1.47 (95% confidence interval 1.16-1.77). Survival to adult was significantly different from the control only in groups exposed to the 2 highest concentrations tested, averaging 3.9 (1.4-9.6) and 8.7 (1.6-20.0) % WSF.

c. There was a distinct decrease in the measured level of jet fuel in the flow through system throughout this test (Figure 20). These declines are probably the result of increasing bacterial populations capable of degrading the

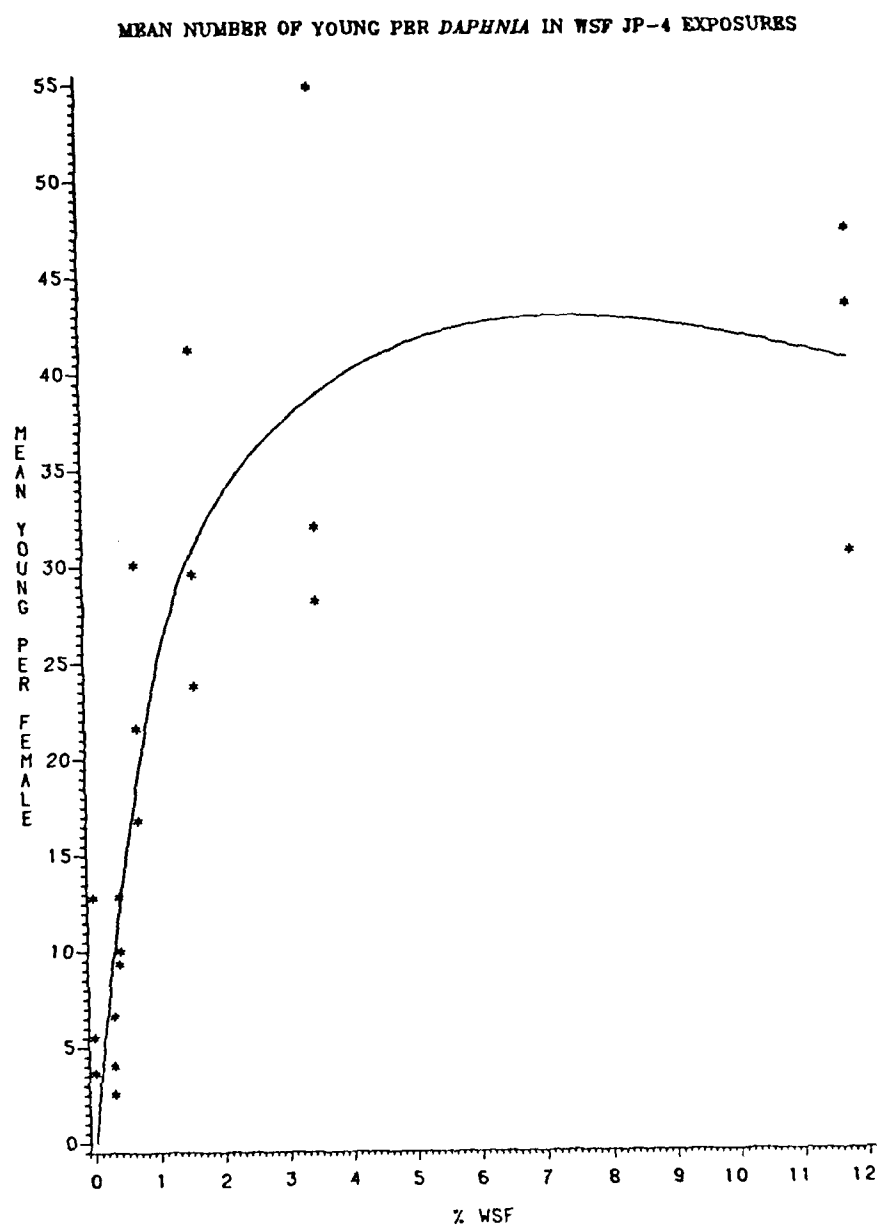


Figure 19: Reproduction of Daphnia pulex in WSF JP-4 exposure.

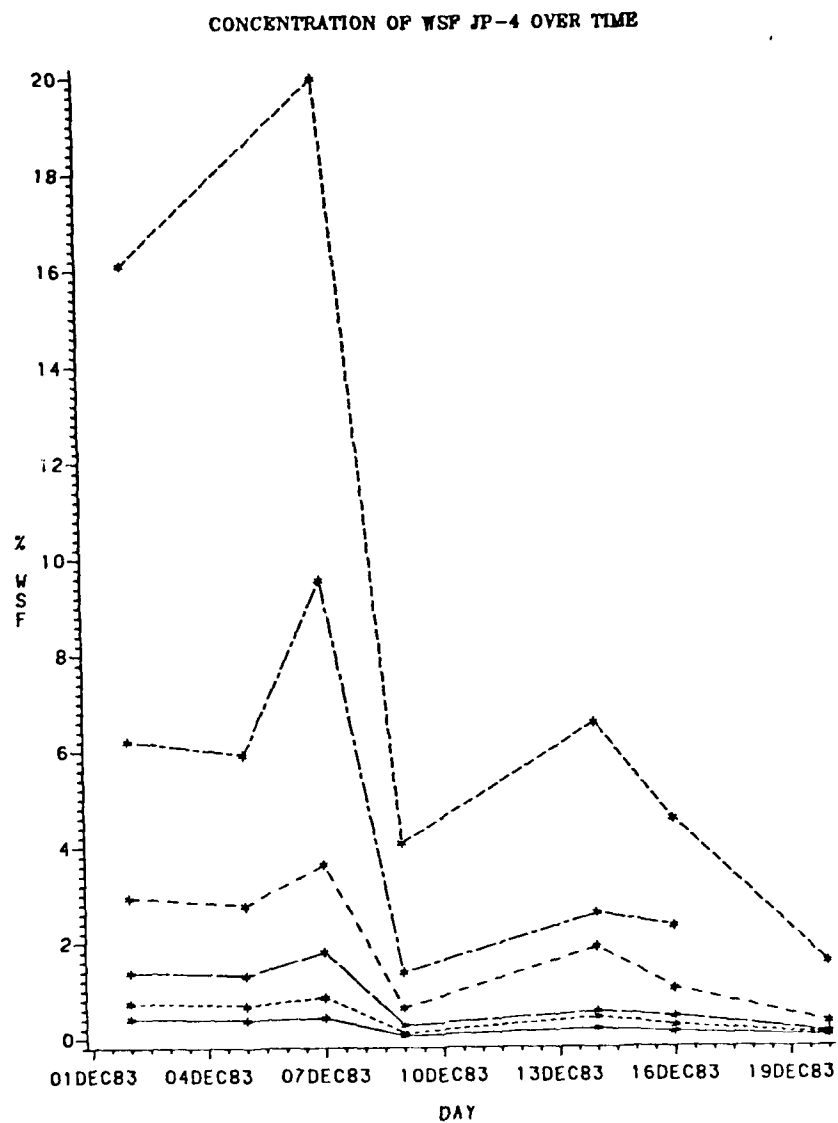


Figure 20: Measured concentrations of WSF JP-4 in a flowthrough test with Paratanytarus parthenogenica.

benzene and toluene components of the WSF. The data from this test are still being analyzed.

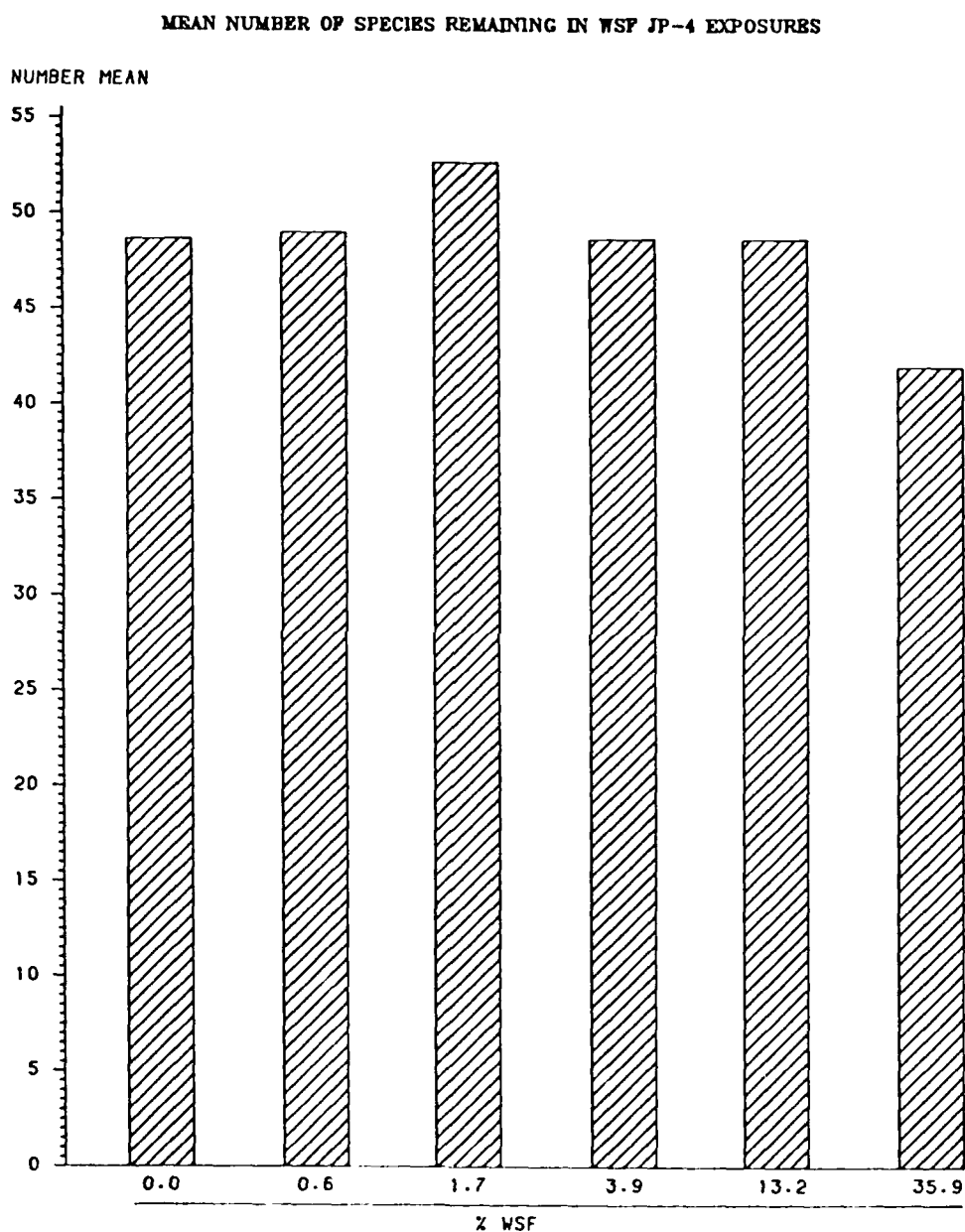
C. Microbial Communities

1. Acute Test-- There were no significant differences in the number of species remaining on PF substrates after a two day exposure to WSF (Figure 21). The highest concentration attained, 35.9 % WSF only had 14 % fewer species than the control.

2. Chronic Test-- In the preliminary chronic test, WSF seemed to increase the colonization rate of barren substrates but did not affect the equilibrium number of species (Table 6). More data is needed before specific comparisons to the control can be made.

III. Conclusions

Results to date suggest that there is relatively little acute toxicity of petroleum jet fuel WSF to two species of aquatic macro-invertebrates or to microbial communities even at the highest concentrations obtainable. In contrast, the aquatic insect, Paratanytarsus parthenogenica, was sensitive to acute exposures with an LC50 of 2.2 % WSF. Chronic tests with invertebrates and microbial communities produced effects of exposure at concentrations ranging from 0.3 to 8.9 %. Additional tests will clarify the range of chronic toxicity.



MEANS CONNECTED BY THE SAME LINE WERE NOT SIGNIFICANTLY DIFFERENT ($P=0.05$)

Figure 21: Effects of WSP JP-4 exposure on a number of species in microbial communities.

Table 6: Effects of petroleum jet fuel WSF on colonization in microbial communities.

Concentration	Colonization Rate	Equilibrium Number of Species	Maximum Observed Number of Species	Number of Species on Epicenter
Control	0.46	30.7	28	37
0.2	0.94	22.5	25	25
0.5	2.14	24.8	26	25
1.2	1.04	29.1	32	28
4.0	1.08	27.6	29	19
9.7	-----*	-----*	29	19
25.6	-----*	-----*	23	23

* The data did not fit the model. No estimates could be obtained.

PART IV

I. ADMINISTRATIVE

A. Personnel

1. Dr John Cairns, Jr., University Distinguished Professor and Director of the UCES serves as Principal Investigator and Dr Arthur Buikema, Jr., Professor of Biology, serves as Senior Investigator for this project.

2. The primary researcher on this project is Major Thomas R. Doane, a graduate student working on a PhD in Zoology in the Department of Biology. He started at VPI&SU in July 1981 and has been working on this project since that time. His education is being paid for by the Air Force Institute of Technology (AFIT). He does not receive any financial assistance from this grant.

3. The two full time technicians working on this project this year were Ms Barbara Neiderlehner and Mrs Sylvia Sanford.

4. Other part time personnel working on this project have included Dr David Stetler, an Associate Professor of Biology, and Mr Brewer Pedin, an undergraduate at VPI&SU. Mr George Schupin has done much of the electron microscopy work although not a part of the supported personnel. There have also been several other graduate students who have assisted and been paid on an hourly basis as have some secretarial personnel.

B. Presentations

1. Major Doane presented a paper at the Virginia Academy of Science on the results to date on the sublethal effects of the WSF JP-4 on the bluegill in May 1983.

2. Major Doane also presented a poster session at the Society of Environmental Chemistry and Toxicology in November 1983. This poster presented all the data on the bluegill research up to that date.

II. Research Progress

A. The original research objectives were:

1. Determine the acute and chronic toxicity of the water soluble fraction of a jet fuel (WSF JP-4) to the freshwater bluegill, (Lepomis macrochirus) after conventional and episodic dosing.

2. Determine the effects of sublethal concentrations of WSF JP-4 on fish ventilation rates.

3. Determine the effects of sublethal concentrations of WSF JP-4 on fish preference/avoidance behavior.

4. Determine the effects of sublethal concentrations of WSF JP-4 on fish blood chemistry.

5. Determine the effects of sublethal concentrations of WSF JP-4 on selected fish tissues.

6. Determine the acute and sublethal effects of WSF JP-4 on selected aquatic invertebrates.

7. Determine the effects of WSF JP-4 on microbial communities.

8. Compare the data collected for the objectives outlined

above and determine if capabilities exist for predicting sublethal effects of stress from the parameters measured.

B. All of the work on the sublethal effects of the petroleum derived JP-4 jet fuel for objectives 1. through 5. have been completed with the exception of the chronic studies. More detailed study of the histology is needed.

C. All of the work on the shale derived JP-4 jet fuel will be completed in this last year. As all of the equipment has been constructed or modified and all experimental procedures have been developed this portion of the research will procede rapidly.

III. Budget

The financial report has already been submitted by the VPI&SU Office of Funded Research.

IV. If there are any questions on this report please contact:

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